

Living with No-growth

THERE has been a remarkable transition in the past few weeks from a situation in which energy was a nagging middle term concern, to be dealt with by orderly re-adjustment of priorities, to that which prevails now, in which fuel shortages are commonplace and even desperate measures may be ineffectual. The consequences of this sudden crisis can barely be guessed yet, because although it is indisputable that oil prices in the future will be much higher, it is an open question whether oil will ever flow as freely again. The view from Britain is bound to be coloured by the simultaneous progressive seizing up of the electricity and coal industries and the railways on account of industrial action. Nevertheless the situation can hardly be said to be other than gloomy in any industrialised society.

The cutback in oil supplies has some advantages to it. At a stroke, it highlights the extraordinary way in which we have been acquiring energy cheaply and squandering it. Certainly no government would have been able to impose restrictions on home heating, office lighting, high speed driving, flying half-empty aircraft and sundry other current afflictions in the absence of such a visible crisis. Yet now there is little complaint at such impositions and no doubt that such moderation in our habits will become part of our way of life.

Further lessons in restraint should also be sinking in. Military ventures by countries less than self-sufficient in oil and capable of supplying the huge increase in demand that war generates will be out of the question. This may not hold back the Soviet Union, but the United States, which has allowed itself to be lured by the deception of cheap energy into becoming an importer of fuel despite its huge resources, must temper its military policies unless it wants the North-east to freeze to death.

It is necessary to think beyond the immediate problems, however, and there is little doubt that the impact on science of the discontinuous change in the economic situation will be very serious. Economic growth for 1974 has been abandoned, implicitly if not explicitly, in many countries. A reduction in oil supplies, even temporary, makes even a stationary gross national product a difficult target to reach.

For a long time economists have debated the pros and cons of a no-growth economy and the arguments in favour of such a concept have become more appealing, if not yet compelling, in recent years. The Fall 1973 issue of *Daedalus* is devoted to a scholarly debate on the subject, which makes for interesting reading now that no-growth is thrust upon us, rather than, as even its keenest advocates would have, being introduced over a space of years.

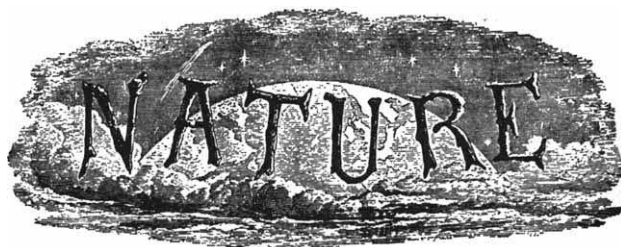
Growth, as Harvey Brooks puts it, "provides opportunities for talented or ambitious people to rise in social status and income without depriving others of status". If there is no growth then nobody can prosper without another faltering and there is palpably a great social need to ensure that if there is to be any income redistribution it is such that those at the bottom gain at the expense

of those at the top. But what is true for individuals is also true for institutions. Any spell of no-growth will at best freeze government spending and may well reduce it substantially, as such spending is an easily manipulated economic tool. It is very difficult to see how science budgets strongly dependent on government funds could remain intact under these circumstances. No-growth could never produce an Apollo programme, indeed Mancur Olson goes even further, "there would be few if any frontiers . . . in a no-growth society".

The problem is not purely a British one, although Britain in its present predicament would feel the effects of an unplanned stoppage of growth most keenly and most quickly. All industrial societies with growth targets that now have to be lowered will experience severe conflicts over priorities, and science is likely to come out the loser, especially when in competition with schools and hospitals for the same money. Such a situation could presumably render a large number of scientists redundant. A preferable alternative is that governments will see that even if they cannot hold the line on pure science they can justifiably switch scientific manpower to social needs programmes.

If the situation is not good in industrial countries, it is little short of appalling in the developing world. Even for those countries that the producers exempt from oil cuts, the rise in the price of oil must do damage to economies at a time when the prospects for improved aid from richer countries are negligible. It is not out of place to question whether science and scientific institutions can survive at all in developing countries.

100 Years Ago



THE Council of the Society of Arts have given notice of their intention to provide a short course of lectures suitable for a juvenile auditory during the Christmas holidays. For this purpose arrangements have been made with Mr. Frank Buckland, M.A., Her Majesty's Inspector of Salmon Fisheries, to deliver two lectures "On the Structure and Habits of Beasts, Birds, and Fishes, as showing Beauty and Design," on Friday, January 2, and Friday, January 9, at 8 P.M. The lectures will be illustrated by specimens. It is intended to make every effort to obtain an entirely juvenile audience, and the notice in the Society's *Journal* impresses strongly upon the members the fact that only children, not adults, are wanted.

From *Nature*, 9, 151, December 25, 1873.



World Health Organization and Genetic Disorders

In *Nature* of June 22 (243, 433; 1973) Professor J. H. Edwards of the Department of Human Genetics at the University of Birmingham commented on the World Health Organization Report Number 497 on *Genetic Disorders: Prevention, Treatment and Rehabilitation*. In this article the group which prepared the report responds to the criticisms of Professor Edwards, who replies.

PROFESSOR J. H. EDWARDS'S quixotic commentary on the WHO report on the prevention, treatment and rehabilitation of genetic disorders should be corrected for the record.

He objects to the first paragraph of the report which refers to the "relative increase in morbidity and mortality attributable to [genetic factors resulting from] slow changes in gene frequencies". The phrase was quoted incorrectly (the portion in brackets was omitted) and out of context by Professor Edwards; it is one of three phrases which briefly introduce the concept that genetic disease is now of greater importance compared with its relative insignificance in the preceding decades of medical practice. Moreover, it would seem implicit that, if genetic disorders are diagnosed and treated in such a way as to alter fitness to any degree, gene frequencies will gradually reach a new equilibrium in man.

Reliability

Professor Edwards does not accept the statement that there is "no reliable information on the current prevalence of genetic disease", a phrase his reader thus sees in isolation because he again quotes out of context. The pertinent paragraph in the WHO report adumbrates: "or on the effects that treatment and prevention will have on the number of cases; there are problems that now need to be studied. Frequencies of individual traits will vary with geographic region, ethnic group, isolate, and deme. The surveys must be both general and particular, and their findings will have both broad and specific objectives. Accurate knowledge about the epidemiology of genetic disease is an important prerequisite of action by a particular community concerning prevention, treatment, and rehabilitation". It was the concern of our group that general information on prevalence of genetic disease, some of which is cited in Tables 2, 3 and 4 of Annex 1 to the report, should not be extrapolated uncritically to specific communities where

such data may not be currently available. Professor Edwards then declares "in fact, the (current) information is adequate for practical purposes; the numbers of the deaf, the blind, and the ineducable are known in all countries with compulsory education, and the proportions of these due to Mendelian and chromosomal disorders have been estimated on good data". Surely Professor Edwards does not believe that the deaf, blind and ineducable represent the only genetic diseases that need to be considered 'for practical purposes', and that compulsory education is the only bench mark of concern for his fellow man.

Meaning

Professor Edwards says that "the word genic is extended from its regular use to include multifactorially determined disorders". Multifactorial disorders are indeed genic, at least in the component which is genetically determined, he would surely agree! The concept of multifactorial disease as we described it was carefully defined but he has chosen not to quote those portions of the report. In the same paragraph, he refers to "a table on multifactorial disorders" in which "congenital malformations and conditions of unknown and non-genetic disease are used as exclusive categories". There is no such table in the report. If he had been accurate and referred his readers to Table 1 of Annex 1, which described the frequency of genetic disorders among patients admitted to a paediatric hospital, they would find that the categories therein are not intended to be exclusive. On the preceding page, it was explained that the classification "unknown" refers to: hospital admissions, not classifiable according to the McKusick catalogue of Mendelian inheritance in man (autosomal recessive, autosomal dominant, and X-linked disease), or the current classifications of chromosomal disease, or the NIH catalogue of congenital malformations; one third of which may possibly be hereditary conditions. On

this basis, we must disagree with Professor Edwards that "multifactorial" means the same thing as "of unknown aetiology". We are aware of Professor Edwards's views on the subject; unfortunately he did not tell his readers the difference between his views and our specific use of simple and carefully defined terms.

On page 31 of the WHO report, a mutation rate of 10^{-4} is used to demonstrate a very slow doubling time for the frequency of a rare recessive gene even with such an enormous mutation rate. It is quite clear the report did not intend the reader to assume that mutation rates in man are uniformly of this magnitude. Professor Edwards's carping at this point, taken out of context, is again misleading and unfair to his readers.

It is false for Professor Edwards to say: "genetic counselling urging couples (heterozygous for deleterious recessive genes) to total abstinence from reproduction is recommended". On page 33 of the report, it merely says that this procedure would be a slightly more effective method for high-risk patients to reduce the frequency of genetic disease than prenatal diagnosis with selective termination of pregnancy. Furthermore, the report states (page 34) "in the case of rare recessive genes, artificial selection against heterozygotes would be folly since every zygote is estimated to carry one or two deleterious genes". It is surprising that Professor Edwards then associates the former comment with the fact that almost everybody is a carrier of recessive deleterious alleles. Perhaps he failed to read the report carefully enough to assimilate the latter commentary. Fortunately we are usually carriers of alleles which are different from those of our spouses; only when two spouses are carriers of the same deleterious allele would genetic counselling be relevant, as Professor Edwards very well knows.

Claims

Professor Edwards takes exception to the "remarkable claim that cystic fibrosis (and sickle cell disease) can be diagnosed *in utero*". He is referring to material appearing in a table listed as Annex 2. Again, he cites the above-mentioned items out of context and without reference to the relevant material in the main body of the text (pages 19 to 21) where the appropriate cautions about prenatal diagnosis are stated. Only those hereditary diseases

eligible for prenatal diagnosis at the time the Scientific Group met (November 1971) are listed in the Annex; those which had actually been diagnosed *in utero* are printed in italics. Neither sickle cell disease nor cystic fibrosis is printed in italics; moreover, the former is keyed to a footnote which indicates that, since the condition is known to be genetically heterogeneous, particular care must be taken with those methods which at the time were thought to be of some use in prenatal diagnosis of this particular disease. The literature sources of the material in the table are clearly stated on the same page of the WHO report. Professor Edwards or any careful reader can obtain the details by reading the source material—as we did.

Professor Edwards criticises our use of compound interest to estimate the doubling time for multifactorial disorders, as cited in the final pages of the report (Annex 3). Although he criticises this approach, he does not suggest an alternative. The final graph, showing the time in generations required to double the frequency of harmful genes for single gene diseases, should reassure Professor Edwards rather than alarm him; doubling times of 1,000 or 100 or even 10 generations would hardly seem alarming, especially for frequencies so minute.

While we are waiting for Professor Edwards's precious grail of "orderly development of either practice or theory" to be found, we hope that he will permit those who have access to the report to use the great part of its 46 pages which escaped unscathed from his comments, for the purposes intended, namely, to begin to apply current genetic knowledge to the immediate benefit of the patient with genetic disease.

Error

We, "an assembly of experts with a dedicated secretariat . . . appropriately stratified by hemisphere, power block and language", remain grateful to Professor Edwards for pointing out an error in the report. We stated that relaxed selection, acting over 100 generations, would increase the frequency of a trait from 0.001 to 0.0086; the correct final frequency should be 0.0102. We fail, however, to see how this or any other alleged error in the report affected the conclusions that were drawn by the committee. If, as Professor Edwards asserts, "some" of the "doubtful assumptions, erroneous facts and uncertain inferences" he finds in the report "overflow into recommendations", would it be too much to ask how correction of a single one of the "errors" he cites would, or should, have altered the recommendations the committee made?

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PROFESSOR EDWARDS writes: Quotations are by nature out of context, and any obtuseness in understanding that small fraction of this technical report which was not technical, and which, rightly or wrongly, I considered could lead to an exaggerated claim of the need for counselling, must be left to the reader. So far as Annex 1 goes it seems reasonable to regard categories specified by percentages which sum to 100% as exclusive. In Annex 2 the heading "All the diseases in the following list can now be diagnosed prenatally" also seems clear. I was certainly in error in overlooking the conditional following the unfortunate apposition of "counsel" and "urge".

I do not think I am alone in feeling some anxiety at the casualness with which genetic counselling is being advanced as a salve for the problems of either our natural variability or for the therapeutic intractability of some of its consequences and, where emotion and enthusiasm run high, there is no substitute for precision in word and number and caution in application. The greater the expertness of a committee, or the weight of authority of the organisation, or its area of influence, or the distinction of its previous reports, the greater the need to expose to public accountancy any doubtful claims which may be influential. I am not doubting the benefits available to the populations of, for example, Montreal or Moscow, or the advantages of their wider availability, provided that they are subordinated to an efficient administration for diagnosis and therapy and do not compete with this basic requirement in the allocation of funds. I am doubting the scale of benefits to populations of counselling *per se* and disturbed at the casual widening in terminology by which counselling is extended to diagnosis and unsolicited advice, therapy to selective abortion, genetic disease to multifactorial disease (I hardly claim any originality for the view that all diseases are multifactorial, but some are less multifactorial than others), and prevention to elimination. In neither Montreal nor Moscow, nor in any other city, would I wish to see unsolicited genetic counselling being given on the basis of computerised files of so-called multifactorial disease (which includes schizophrenia) in relatives. I do not share the optimism of the group which "is convinced that genetic registries can be designed and used in ways that will contribute to the diagnosis and prevention of genetic disease without

endangering individual rights", unless genetic is used in the restricted sense which excludes multifactorial, or the registries are decentralised and maintained within hospital records departments. Quixotry implies an aggressive and well-intended reaction to a delusion and, while I hope my anxieties are delusions, I remain unconvinced.

It would be a great tragedy if drawing attention to any apparent *non sequiturs* should in any way be regarded as critical of the technical reports in general, or of the administration under which they are edited. Effective documents are necessarily controversial and the genetic future of man is too important a subject to be constrained by the niceties of private dissent.

BIRTH DEFECTS

Hope for Spina Bifida

SINCE the publication of the WHO report number 497 there have been several advances in the prediction in the uterus of a defective embryo. Perhaps the most significant of these has been the discovery that foetuses which are anencephalic or which suffer from spina bifida release a high amount of alpha foetoprotein into the amniotic fluid. This high protein level, if detected as a result of amniocentesis, is a sign of a deformed foetus.

In Britain about 2,000 anencephalic children, and a similar number of spina bifida cases are born annually. In principle these births can now be avoided if a positive amniocentesis is followed by abortion.

It is, however, impracticable to study every pregnancy in Britain (731,000 births in 1972). Therefore the discovery by Dr D. J. H. Brock and colleagues at the University of Edinburgh (*Lancet*, ii, 923; 1973) that for anencephaly an excess of alpha foetoprotein is found in maternal blood, is welcome.

Spina bifida, however, throws up greater problems than anencephaly for such children who undergo surgery soon after birth, live for a long time, although they are usually both physically and mentally handicapped afterwards. Anencephalic children die at birth.

An excess of alpha foetoprotein is also found in the blood of some mothers who are carrying spina bifida foetuses but Dr Brock said this week that the test on maternal blood was not as reliable as in the case of anencephaly. It seems that an excess of protein is found when the foetus is very deformed but when the deformation is slight there is not always a detectable amount of the protein in the blood.

NEWS

Nuclear Weapons in Europe

by our Washington Correspondent

A RARE glimpse of the extent and nature of the nuclear umbrella that the United States has erected over Europe is provided in a report prepared by staff members of the Senate Foreign Relations Committee. It indicates, among other things, that as many as 7,000 nuclear warheads are stockpiled in more than 100 special ammunition sites throughout Europe and, although all the weapons are in United States custody, about two-thirds of them are for use by European forces. Some of the weapons are constantly loaded, ready to be unleashed on command.

The report has been heavily censored by the Department of Defense and it is tantalisingly devoid of detail. Nevertheless, it lifts a fraction of the veil of secrecy that surrounds European nuclear defence planning and it brings together—and confers official status on—information which has previously appeared unofficially in the public record. It also emphasises the extent to which Europe is dependent on the United States for its defence.

Although delivery systems capable of carrying nuclear weapons were deployed by the United States in Europe during the 1950s, warheads were not stockpiled until President Eisenhower formally proposed the idea at a NATO Heads of Government meeting in 1957. Since then, the buildup of American nuclear weapons in European bases has been governed by a set of bilateral agreements between the United States and individual European governments. One of the agreements provides that the location of storage sites is to be determined by the NATO Supreme Allied Commander in Europe (SACEUR)—who is also commander in chief of American forces in Europe—in agreement with United States military authorities and the government of the country concerned. The agreements also specify that the weapons will remain in the custody of United States personnel, and that the 'user country' will pay the costs of stockpiling them.

The bilateral agreements are broad and they do not identify individual nuclear weapons systems. In fact, the Senate committee report states that it was not until 1967—a full ten years after stockpiling began—that the United States finally agreed to provide Euro-

pean governments with details of the nuclear weapons stockpiled in their territory or stored elsewhere for use of their troops. (That revelation begs the question of how NATO managed to carry out sensible defence planning without knowing the nature of the weapons that the United States had installed in Europe.)

Robert S. McNamara, Secretary of Defense, announced in 1966 that there were then some 7,000 nuclear warheads stockpiled in Europe, a figure which the report notes "was the first, last and only official revelation regarding the size and disposition of the American nuclear armory in Europe". The total has since fluctuated and the present level has been deleted from the report, but 80% of the land-based warheads are for tactical weapons which can be used for offence or defence and the rest are purely defensive.

The warheads are stored in special

igloos, in sites surrounded by double fences which are floodlit at night. Some of the weapons, including a proportion of those for use by European forces, are always maintained on what is termed quick reaction alert, ready to be fired as soon as the order is given. The proportion of the total nuclear force which is ready to be unleashed depends on the general state of alert in the NATO forces.

An agreement drawn up by NATO members in 1969 provides for consultation among member governments before nuclear weapons are used in Europe. According to the Senate report, it would work like this. A request for the use of nuclear weapons in the defence of NATO, which comes from either a member government or from a major NATO commander, or any possibility that a nuclear power may use nuclear weapons to defend NATO, will be communicated immediately to all NATO

RESEARCH SPENDING

Battelle Forecast of Increase

by our Washington Correspondent

ESTIMATING the amount of money that will be spent on research and development in 1974 is a risky business, particularly in the light of the great uncertainties in the economic outlook. Nevertheless, according to the annual forecast of the Battelle-Columbus Laboratories, the total amount to be spent on research and development in the United States next year is expected to reach about \$32,400 million—an increase of some \$2,300 million over this year's expenditure. The Battelle forecast is perhaps the most widely expected of all the exercises in crystal-ball gazing that are carried out in the United States to predict funding for research and development.

Although the expected increase would be mostly swallowed up by inflation—which is reckoned to be running at about 5% for research and development costs—it would nevertheless, represent a modest growth in purchasing power. Industry is expected to provide a relatively large increase in research funds, and support from the Federal Government is also expected to grow, according to the report.

Battelle acknowledges, however, that the forecast could be upset by energy shortages and other factors. On the one hand, if the energy crisis leads to widespread recession, industrial research and development could be hard hit. But, on the other, the energy crisis is likely to stimulate increased research and development on energy production and conservation.

The forecast sees the Federal Government providing about \$16,800 million, industry more than \$13,700 million, universities and colleges about \$1,500 million and non-profit institutions about \$480 million. Industry is therefore expected to provide about 42% of the total research and development funding next year, compared with 31% ten years ago. But the increases will not come from every industrial sector, for the forecast predicts that four industrial groups—food manufacturers, chemical companies, instrument makers and producers of electrical machinery—will increase their research and development funding at a rate greater than the rate of inflation. Expenditures by other industry groups will probably not even keep pace with inflation.

governments and to the Defense Planning Committee (a top level committee consisting of all NATO members except France). The views of the individual governments and of the Defense Planning Committee would then be transmitted to the nuclear power concerned. In other words, the Supreme Allied Commander in Europe would "not be permitted to use nuclear weapons unless there were consultations with NATO member governments directly and with NATO itself".

In the final analysis, however, only the President of the United States or, for British nuclear weapons, the British Prime Minister, can authorise the release of nuclear weapons. Thus Presidential approval would be required before any use of United States nuclear weapons by NATO forces.

The Senate report outlines general NATO strategy with respect to both conventional and nuclear forces, the basic elements of which are "nuclear deterrence, forward defense and flexible response". Should deterrence fail, there are three levels of military response open to allied forces. "The first is direct defense—that is, defeating the enemy on the level he chooses to fight, a concept which includes the use of such available nuclear weapons as may be authorised". The second level of response would be "deliberately raising, but where possible controlling, the scope and intensity of combat, making cost and risk disproportionate to the aggressor's objectives and the threat of nuclear response progressively more imminent". The final response would be "massive nuclear strikes against the total nuclear threat, other military targets and urban industrial targets". The ultimate general nuclear response could, however, be carried out only in conjunction with the United States Joint Chiefs of Staff plan for widespread synchronised use of United States nuclear weapons. "Accordingly", the report notes, "responsibility for carrying out NATO's general nuclear response falls on US strategic forces based outside Europe".

EPA

Appearing Objective

by our Washington Correspondent

MR RUSSELL TRAIN, the new Administrator of the United States Environmental Protection Agency (EPA), has decided to cut off a subsidy that the agency provides to an industrial research organisation because of public allegations that the subsidy compromises the EPA's regulation of automobile pollution. The organisation concerned is the Coordinating Research Council-Air Pollution Research Advisory Committee

(CRC-APRAC), a research organisation supported by the automobile industry, the oil industry and the EPA. It has sponsored some of the key research on which many emission control regulations have been based.

The decision to sever ties between the EPA and CRC-APRAC was based on a review conducted by Mr Train shortly after he became administrator of the agency. He explained in a letter to Mr M. K. McLeod, director of CRC-APRAC, that although he is entirely satisfied that the organisation's work has been objective and free from bias, "not only the fact of objectivity but also the appearance of objectivity must be considered when it comes to evaluating continued EPA participation in this activity".

CRC-APRAC receives three-quarters of its money from the American Petroleum Institute and the Motor Vehicles Manufacturing Association, both of which are industrial organisations, and the rest from the EPA. It funds research on the effects on health of automobile emissions, on emission control technology and on vehicle testing.

The chief criticisms of the agency's ties with CRC-APRAC have come from Senator Edmund S. Muskie, Chairman of the Subcommittee on Air and Water Pollution, and the chief sponsor of pollution control measures in the Senate. He has charged that the organisation's advisory committee, on which representatives of the automobile and oil industries predominate, decides, without outside advice, what research should be undertaken; that the research is then monitored by panels which are also dominated by industry representatives; and that the results eventually wind up as the basis for regulations vitally affecting the industries concerned.

As evidence of conflict of interest in CRC-APRAC, Muskie published an internal EPA memorandum in the *Congressional Record* last week which discusses the motor industry's attitudes to the EPA's programme to control emissions from heavy-duty diesel engines. According to the memorandum, an official of Cummins Engine, one of the two largest manufacturers of diesel engines in the United States, said in a discussion that the CRC-APRAC programme to develop new test procedures for heavy-duty engines/vehicles was the most "effective coup that the industry has pulled off on EPA" as it commits the EPA to a long and complicated project which could postpone instituting controls for heavy-duty engines for many years.

In addition to severing its formal ties with CRC-APRAC, the EPA has decided to take over the management and funding of three projects which are now being carried out under the organisation's auspices.

COAL

Aggregated Waste

DAMAGING though the miners' overtime ban may be to the National Coal Board (NCB) there is one section of the board that has its problems temporarily eased. The Minestone Executive is responsible for the 50 million tons of spoil that emerge from the ground each year along with the coal. While the overtime ban is on, the quantities are at least a little smaller.

With the increasing mechanisation of the coal industry the quantities of waste produced have risen sharply. Machine mining often takes parts of the roof and floor away at the same time as the coal and this extra waste travels up to the surface.

But attempts to do anything with the shale that was accumulating in massive tips in the mining areas are relatively recent. Only in the past fifteen years has a serious effort been made. It is estimated currently that the coal board has in the region of 3,000 million tons of spoil lying around Britain.

Now that local authorities have tightened up on their planning procedures new tips are hard to start and old tips are frowned on, so the NCB has the job of tidying up the past and preventing desecration in the future.

Finding uses for the shale has never been easy. Several million tons have been used as fill for motorways. About half a million tons a year have been turned into bricks by the Scottish and the Midland Brick Companies, and some spoil has been turned into a base matrix for the extremely tough but very expensive Guyana bauxites. This mix has a high skid resistance and has been put on Britain's crossroads particularly in the Greater London Council area. But, beneficial though this use is, the tonnages involved are relatively small.

With the large motorway programme in the coalfield areas coming to an end the demand for spoil as a motorway fill is decreasing. Although major road works will still need the spoil, the sharper bends and steeper hills allowed on main roads will cut the demand.

But with the oil shortage certain to increase the cost of transport, colliery spoil could benefit. Transport is the highest part of the cost of any shale. With the coal fields in the centre and in the north of Britain it could become economically attractive to use closer, although less satisfactory, shales from collieries than to go further afield for a higher quality stone.

The best future use for shale may lie in the aggregates business. The Verney Committee, set up by the Department of the Environment, produced a preliminary report recently in which it stated that current demand for aggregates is around 220 million tonnes a

year; a figure that will rise to 330 million tonnes a year by 1980.

The committee urged that more research should go into alternative aggregate supplies—which includes the NCB's spoil. The availability of sand and gravel from pits and quarries is becoming more limited—particularly in the south-east—and environmental considerations are coming into play.

As a first step to large scale commercial aggregate production, a half million ton a day plant is being built at Snowdown in Kent.

This aggregate is lightweight material which will probably be used for cladding purposes rather than as structural concrete. The British lightweight aggregate market runs at about 4 million tons per annum at present, but with natural aggregate becoming scarcer and more expensive there may well be a larger market available to the board in the manufactured aggregate field.

If it were decided to get rid of the spoil heaps by any available method, the board has calculated that there is five times as much space available in quarries as there is spoil on the surface.

Short Notes

Another Leap Second

EVIDENCE that the Earth continues to run slow comes from the Time Department of the Royal Greenwich Observatory, which has announced that the atomic time signals will be retarded by 1 second at midnight on December 31. This is done by inserting a so-called time step, or leap second, into the time signals so that the familiar BBC six-pip signal becomes seven pips.

This is the third time step that has had to be added since the atomic standard was introduced at the start of 1972. By agreement, the atomic time signals are not normally allowed to diverge from universal time (UT or GMT) by more than 0.7 s.

Currently, the Earth loses about 3 ms a day on Atomic Time, and the leap seconds are added where necessary—preferably at the end of June and/or December—to keep UT and Atomic Time in close correspondence.

By the end of December the Atomic Time signal will be 0.3 s ahead of UT. After the time step, Atomic Time will lag UT by 0.7 s. When the next time step has to be introduced will depend on how UT flips back with respect to Atomic Time during 1974.

Prostaglandin on the US Market

THIRTEEN months after its introduction in the United Kingdom, a prostaglandin has become commercially available on a similar basis in the United States. The United States Food and Drug Admini-

stration has permitted limited distribution to hospitals and clinics of Prostin $F_{2\alpha}$, known in the laboratory as prostaglandin $F_{2\alpha}$, one of the family of compounds considered to hold promise for treatment of such conditions as asthma, ulcers and high blood pressure, as well as for their ability to induce uterine contractions. The drug, developed and produced by the Upjohn Co., Kalamazoo, Michigan, will be used specifically for the induction of abortion during the second trimester of pregnancy—the fourth to sixth month. At this stage Prostin $F_{2\alpha}$ will be sold only to university medical centres where it has already been used in research or where physicians are familiar with the techniques involved in its administration. These presumably comprise chiefly the obstetricians who gathered at Upjohn's conference centre, Brook Lodge, in Michigan, in June 1972 to discuss the progress of their clinical trials (*Nature*, **237**, 482; 1972). At that time the most satisfactory mode of administration during the second trimester seemed to be direct injection into the amniotic sac and Prostin $F_{2\alpha}$ has been produced in a form suitable for use in this way.

Artistic Talent

THE Medical Art Society is extending an invitation to scientists who sculpt or paint to exhibit with the society at its exhibition in London in 1974.

Scientists are particularly invited to contribute to the section of the exhibition called "intraism". The society describes what this means as follows. "Portrayal of the 'hidden' and portrayal of the 'minutiae' are the goals we of this century can achieve in the three modalities of representation termable intra-realism, intra-impressionism and intra-abstraction".

As a further explanation for the uninitiated, intraism is described as an art form which "draws on psychedelic thought and seeks the art description of the structural facts science, biology and medicine collate for us, concerning the regions within the body surface, within its substance and within its tracts, channels and impouchings".

Suitably motivated readers of *Nature* can now proceed to buy their canvasses and clean their brushes.

Naming Planetoids

THREE minor planets recently discovered by the Crimean astrophysical observatory have been named Dobrovolskii, Volkov and Patsaev, after the cosmonauts who perished in the Soyuz-11 disaster. This breach of the nomenclature rule that planetoids in the belt between Mars and Jupiter should bear feminine names is extremely rare for Soviet-named planetoids. Even so eminent a

personage as Tsiolkovskii required a change of gender before being commemorated as "Tsiolkovskaya", and planetoid 852, the first discovery of the Soviet era, commemorates Vladimir I. Lenin in the feminine guise of "Vladilena".

Since the Revolution, Soviet planetary nomenclature has been under the control of Professor Natal'ya Sergeevna Yakhontova, a pioneer of equal rights for women in astronomy.

Predicting Population

MANY people have come to grief in trying to make predictions of the future population. One of the chief problems is that fertility is most quixotic and it is not possible to tell in advance how this is going to change. As an example, nine months after the great blackout on the eastern seaboard of the United States in 1967 there was an unprecedented increase in the birth rate which made nonsense of all predictions.

What chance is there that the birth rate in Britain in 1974 will register an increase? In 1972 the rate was 14.9 per thousand of the population compared with 16.2 in 1971. Television, it must be remembered, went off the air at 10.30 p.m. from December 17 onwards. In contrast to the situation in the United States in 1967, this will not happen for one night only in Britain. So, look out all maternity units from next September on.

Supersonics 1,000,000—Cloth Caps 0

THE British parliament was last week assured that the Concorde development programme would not be impeded by fuel shortages. Rather over a million gallons of fuel will be needed for Concorde in the next six months, but this is only 0.7% of total aviation fuel requirements.

On the other hand, in the same week the government could see no way that the few British soccer clubs who have bought petrol generators could be allowed to use them for floodlighting grounds. Re-timed fixtures will, at a guess, prevent a hundred thousand from attending each week. A generator uses 20 gallons during a game.

Reverse Energy

Two weeks ago we reported on research in progress in Britain on synthesising gas from coal with the same burning characteristics as natural gas (*Nature*, **246**, 326; 1973).

This week we have been searching for evidence of some research in progress on convertors of natural gas into coal. Unfortunately there does not seem to be any going on—at least in Britain.

NEWS AND VIEWS

Wanted—A Taxonomist for Magnetism

UNTIL quite recently, magnetism was a nice, tidy branch of physics. Every student of the field knew that it was neatly divided into diamagnetism, paramagnetism, ferromagnetism, ferrimagnetism and antiferromagnetism: Néel was in his haven and all was right with the world. Such a sense of tidiness is apt to precede a thoroughgoing shakeup, and magnetism has been no exception. Now there is mictomagnetism, (crystalline) superparamagnetism, amorphous antiferromagnetism and, as the most recent candidate, speromagnetism. What this amounts to is that magnetism has come face to face with the physics of randomness, and randomness has turned out to be more subtle than mere regularity.

'Speromagnetism' is a term coined by Coey of Grenoble and Readman of Edinburgh in their letter on page 476 of this issue of *Nature*. They report on an examination of a natural gel consisting of hydrated amorphous ferric hydroxide in an organic matrix. The magnetisation curve at low temperatures shows a remanence, which indicates that there must be some kind of ordered magnetic structure. Combining magnetisation studies with a detailed examination by Mössbauer spectroscopy, the authors conclude that below 10 K the spins are coupled and locked within small particles of the order of 10 nm in size; above 10 K these particles become 'unblocked' and behave superparamagnetically—that is, the particles are now magnetically independent but in each the spins are still fixed in the absence of a constraining field. Only above 100 K does the material become a self-respecting paramagnet, with thermally randomised spins.

Applying Langevin theory to the superparamagnetic magnetisation curve leads to a net moment of $73 \mu_B$ per particle, and also to an estimate of the particle size as equivalent to 635 formula units of the hydrated ferric hydroxide. This at once poses a paradox, because $73 \mu_B$ for 635 formula units is consistent neither with ferromagnetic nor with antiferromagnetic coupling within a particle. The authors are driven to the conclusion that

the spins are arranged effectively at random. The point to notice is that a purely random array of N spins of moment m in an independent small particle has a resultant moment of $\sqrt{Nm}$: this is simply the random walk equation, familiar from diffusion theory. Although the authors do not trouble to spell it out, the resultant saturation magnetisation is much greater for a population of spins distributed in many minute particles than for the same population arranged in a few large particles.

The authors call the behaviour of an array of small particles with fixed but random spins, which have an appreciable resultant moment only because of the small size of the array, by the name speromagnetism, from a Greek root meaning 'to scatter'. The interesting question is why an array of ferric spins is arranged randomly in spite of the undoubted presence of some antiferromagnetic nearest-neighbour interactions, and on this they are able only to speculate. It seems that the existence of variable exchange interactions in an irregular structure must in some way be held responsible.

This intriguing paper links with a substantial body of recent research concerned with the magnetic properties of extensive (rather than particulate) amorphous materials, dignified this year with a full-scale volume of conference proceedings (*Amorphous Magnetism*, edited by H. O. Hooper and A. M. de Graaf, Plenum, New York). Both ferromagnetic and (even more surprisingly) antiferromagnetic characteristics have been observed in alloy glasses. The theory of spin assemblies where the strength and even the sign of exchange interaction vary according to the spacing of randomly packed neighbours is very complex and has only recently been attempted. The new phenomenon of speromagnetism (or amorphous superparamagnetism) adds a further delectable layer of complexity to what is already a highly elaborate network of phenomena. Truly the services of a magnetic taxonomist will soon be needed to bring order out of the amorphous multiplicity of concepts.

R. W. C.

Prospects for Hepatitis B Vaccines

DISCUSSIONS on active immunisation against hepatitis B infection centre around the work of Krugman and his associates at the Willowbrook State School who use as the 'vaccine' a known infective human serum (MS-2 serum) that contains hepatitis B antigen and which is diluted 1 in 10 and boiled for 1 minute (Krugman *et al.*, *New Engl. J. Med.*, **288**, 755; 1973). This heat-inactivated serum is not infective and it successfully prevented or modified hepatitis B in twenty (69%) of twenty-nine children who were challenged after 4 to 8 months with serum containing the MS-2 strain of hepatitis B virus. The illness in the remaining nine children was generally mild, with jaundice occurring in one child. Abnormal levels of serum glutamic oxaloacetic transaminase were noted in twelve (41%) of the twenty-nine children. Hepatitis B antigen was detected in the serum of twelve children and the

antigen persisted in three of the twenty-nine children giving a chronic carrier rate of about 10%; whereas in a control group of twenty-four non-immunised children a chronic carrier rate of 37.5% was observed after exposure to MS-2 serum. Hepatitis B antibody, assayed by sensitive techniques, was induced in eight of twenty-nine children, but it was transient in six of the children and furthermore one of the children with 'vaccine'-induced antibody became a persistent carrier of the antigen after challenge. The results with the heat-inactivated serum were essentially the same after one, two or three inoculations.

Diluted and heat-treated whole serum may be regarded as an inactivated 'vaccine' but it is a crude way of inducing immunity and it is unlikely that such material will be licensed for general use. The repeated failure consistently to grow hepatitis B virus in tissue or organ cultures has

hampered progress towards the development of a safe and effective vaccine. Attention has therefore been directed more recently towards the use of other preparations for active immunisation against type B viral hepatitis.

The close association between hepatitis B antigen and human hepatitis B virus is firmly established (*Bull. Wld Hlth Org.*, **42**, 957; 1970). The morphology of hepatitis B antigen shares a number of features with known viral structure. The principal antigenic constituent is a rather pleomorphic spherical particle measuring about 20 nm in diameter but with a range of 16–25 nm. The presence of tubular forms, with an average diameter of 20 nm and often several hundred nanometres in length, is a characteristic feature. The third type of particle (Dane particle) is also spheroidal, measuring about 42 nm in diameter, with an inner core of about 28 nm in diameter, a 2 nm shell and an outer coat about 7 nm in thickness. After detergent treatment of pellets of hepatitis B antigen obtained by ultracentrifugation of whole serum, the large 42 nm spherical particles separate into an outer coat of antigen and an inner component which is 27 nm in diameter (Almeida *et al.*, *Lancet*, ii, 1225; 1971). The inner component resembles morphologically a rhinovirus. Almeida *et al.* found that antibody present in the serum of patients after recovery from type B hepatitis reacted with the inner component, but not with the outer hepatitis B antigen component, to yield immune aggregates resembling those seen in homogenates of liver taken post-mortem from patients with hepatitis B. Antibody to the inner core was absent from the pre-hepatitis sera from the same convalescent patients. It was suggested that antibody to hepatitis B antigen develops during the course of the infection, but that it is subsequently cleared from the serum with clinical improvement, while a normal immune response is produced to the inner core of the 42 nm particle. These findings have since been confirmed (Barker *et al.*, *J. infect. Dis.*, **127**, 648; 1973; and Hofnagle *et al.*, *Lancet*, ii, 869; 1973) and lend support to the view that the inner component may indeed represent the infectious agent of type B hepatitis, although final confirmation must await the successful cultivation of the infectious agent.

It is uncertain whether hepatitis B antigen represents incomplete virus particles, aggregates of protein subunits, excess virus-coat material, overproduction of unstable virus-like particles or a modified cellular component whose synthesis is specified or specifically derepressed by the hepatitis B virus. But it seems likely that the antigen consists principally of excess viral coat proteins coded by the infecting virus. Isolated viral coat protein challenges the body's immune mechanism in the same way as the whole infectious agent and the possibility of using purified hepatitis B antigen particles, which are free of nucleic acid and therefore of infectivity, seems attractive. Such an approach, however, is precluded by the amounts of host protein that may be complexed with the viral protein in quantities which are far in excess of the protein coat of most recognised viruses. These host proteins may include various pre-existing structures of the liver cell (Popper and MacKay, *Lancet*, i, 1161; 1972) and may thus induce undesirable immunological reactions. Subunits of the antigen, in the form of small polypeptides, on the other hand, offer a much more attractive proposition as immunogens, although it remains to be determined whether antibody to hepatitis B antigen, to the inner core or to other components is responsible for protection against infection.

Subunit Hepatitis B Vaccine

Hepatitis B antigen was recently fractionated by isopycnic centrifugation in a continuous linear gradient of caesium chloride followed by rate sedimentation separation (Vyas *et al.*, *J. Immunol.*, **108**, 1114; 1972). The rate sedimentation procedure resolved the material into three protein peaks containing the small spherical, tubular forms and the 42 nm (Dane) particles of the antigen respectively. Amino acid analysis of the first peak revealed that the protein seems to consist of two subunits and it contains a high proportion of proline, leucine and serine and a low content of tyrosine.

In another study, Dreesman and colleagues considered some of the biophysical and biochemical properties of hepatitis B antigen in plasma collected from three patients with anicteric hepatitis. The original samples were found by electron microscopy to contain the small spherical 20 nm particles, tubular forms and 42 nm particles. High speed centrifugation yielded pellets which were treated at pH 2.4 before further purification by isopycnic and rate zonal centrifugation. Electron microscopic examination of the pelleted particles after low pH treatment disclosed that both the large 42 nm particles and tubular forms were lost, yielding a preparation containing a mixture of spherical particles with a diameter of 19 ± 2 nm and a population of larger particles measuring 25 ± 2 nm in diameter. The relative proportions of the 19 nm and 25 nm particles were dependent on the original plasma. In one plasma, in which relatively few 42 nm particles were found in the starting material, 90% of the purified particles had a diameter of 19 nm. A plasma rich in 42 nm particles, however, yielded a purified preparation containing 65% of 25 nm particles, which may represent the core material of the 42 nm particles. The difference in size was confirmed by the presence of two distinct populations with average molecular weights, calculated from high speed equilibrium data, of 3.6×10^6 and 4.5×10^6 .

There were no significant differences in the amino acid composition of antigen purified from the plasma of the three patients. A comparison of the amino acid composition of hepatitis B antigen with that of adenovirus type 2, poliovirus, herpes virus and KB cells revealed that the antigen is distinctive in having significantly higher levels of cysteine (or cystine), proline, leucine and phenylalanine and lower levels of both the acidic and basic amino acids lysine, arginine, aspartic acid and glutamic acid. A relatively low concentration of tyrosine and histidine was found.

Six iodinated polypeptides were identified in purified hepatitis B antigen preparations by disk polyacrylamide gel electrophoresis in sodium dodecyl sulphate-urea gels (Dreesman *et al.*, *J. Virol.*, **10**, 469; 1972). The three large polypeptides had molecular weights of 39,000, 32,000 and 27,000 respectively and the three smaller polypeptides had molecular weights of 22,000, 16,000 and approximately 10,000 respectively.

The close association of hepatitis B antigen with normal serum components has been an acknowledged difficulty in developing centrifugation techniques for separation of the antigen in pure form from serum. Since there is almost complete recovery of total protein after separation by electrofocusing, this technique was applied for the purification of hepatitis B antigen (Howard and Zuckerman, *J. gen. Virol.*, **20**, 253; 1973). Antigenic activity was found in those bands of serum proteins which possessed isoelectric points over the pH range 4.0 to 7.0. Antigenic

activity was not detected in association with separated gamma globulins, which is in accord with the long epidemiological and clinical experience that gamma globulin is free of the risk of transmitting hepatitis and with the failure to detect hepatitis B antigen, a marker associated with infectivity, by electron microscopy after Cohn fractionation of human plasma known to contain the antigen (Zuckerman *et al.*, *J. clin. Path.*, **24**, 2; 1971). The major portion of serum proteins was removed by gel filtration on Sephadex G200, concentrated, and hepatitis B antigen separated from the remaining unwanted serum protein by isoelectric focusing in a sucrose gradient containing carrier ampholytes (Ampholine) at a final concentration of 1%. Hepatitis B antigen was found to focus in two discrete bands (Howard and Zuckerman, 1973) with isoelectric points 3.65 and 4.33 for the *ad* subtype and 3.95 and 4.90 for the *ay* subtype (Howard and Zuckerman in *Isoelectric Focusing* (ed. by Arbutnot), Butterworth, in the press). Normal serum proteins were not detected in the two bands. It is also interesting to note the similarity of behaviour of the antigen to some plant viruses focused in polyacrylamide gels (Rice and Horst, *Virology*, **49**, 602; 1972).

The apoprotein constituent of hepatitis B antigen was found to be organised into a number of definable polypeptides. Aliquots from peaks I and II of separated antigen activity were solubilised and the resulting protein solution was then subjected to electrophoresis in 10% polyacrylamide gels. After staining with Coomassie Brilliant Blue, eight identical polypeptides were discernible preparations from both peaks. The range of molecular weights was approximately 10,000 to 300,000. Densitometry of the stained gels revealed that at least one polypeptide (MW 100,000) was a major component of the first peak, but it was present in a small quantity only in the second peak. Selective degradation experiments suggest that this polypeptide is a part of the surface structure of the antigen (Howard and Zuckerman, in the press). Analysis of antigen bearing the *ay* subdeterminants by the same technique has not yet been completed.

Such polypeptide preparations should be investigated as potential vaccines for hepatitis B by defining the immunogenic moiety by studies in appropriate nonhuman primates.

Synthetic Hepatitis B Vaccine

An immunochemical study of purified hepatitis B antigen is essential for understanding this unique antigen. Analogous to the TMVP decapeptide, the primary sequence of the haptenic peptide of hepatitis B antigen may provide another approach for a development of a synthetic peptide, which when coupled to a macromolecular carrier could serve as a suitable immunogen. Once detailed data are available on the protein, peptide and amino acid composition of this antigen, it should be possible to define by animal immunisation the moiety responsible for the antigenic activity.

There are reports in the literature to support the feasibility of such an approach. For example, in 1966 Stewart *et al.* (*Biochemistry*, **5**, 3396; 1966) defined the antigenic moiety of TMV protein decapeptide. Arnon and her colleagues (*Proc. natn. Acad. Sci., U.S.A.*, **68**, 1450; 1971) showed that it was possible to use a synthetic macromolecule for eliciting antibodies reacting exclusively with a specific region of a native egg-white lysozyme. This

was achieved by synthesising a particular segment of the enzyme from its amino acid components, attaching the peptide to a synthetic polypeptide carrier and using the conjugate for immunisation. The resulting antibodies reacted with native lysozyme in a unique, conformation-dependent antigenic determinant. Of course, in the case of lysozyme both the amino acid sequence and the three-dimensional structure are known, and the synthesised peptide was designed on the basis of previous information concerning its contribution to the antigenic specificity of the molecule.

A similar approach might be attempted for type B hepatitis, depending largely on the success in elucidating the structure of hepatitis B antigen, since there is little doubt that polypeptides and other moieties such as specific lipoproteins can be attached to a macromolecular carrier (Sela, *Adv. Immunol.*, **5**, 29; 1966) for subsequent immunisation. The prospects of active immunisation against hepatitis B appear brighter.

A. J. Z. and C. R. H.

Origin of Marginal Seas

WITHIN the framework of seafloor spreading and plate tectonics the origin of marginal ocean basins is still something of an enigma. Thus the geological evolution of the Mediterranean, the Gulf of Mexico and the various marginal seas of the northwestern Pacific is currently hotly debated. The evolution of the main ocean basins within the past 60–70 million years is discernible in remarkable detail as a result of the bathymetric expression and magnetic record of spreading about mid-ocean ridge crests. Even details of the geometry and timing of shifts of a spreading centre can be documented for this period of time. The age of older oceanic crust is less readily determined partly because the bathymetric expression has decayed and there are long periods of time during which the Earth's magnetic field did not reverse its polarity, but also because subsequent igneous activity may complicate both the bathymetric and magnetic signatures. Nevertheless the details of Mesozoic ocean floor spreading are gradually emerging and if unexpected in detail are certainly not unexpected in terms of basic principles. With regard to marginal basins, however, basic principles are at stake; some geologists seriously doubt whether these areas, even where they attain oceanic depths, are floored by oceanic crust formed by spreading about a mid-ocean ridge crest.

Many of the marginal seas are bounded wholly or in part by subduction zones (that is, active mountain belts or island arcs) and the origin or initiation of these zones, both in space and time, is fundamental to a clear understanding of the seas themselves. In contrast to mid-ocean ridge crests, whose position relative to continental margins during the past 100–200 m.y. is often well known, the position of the destructional plate margins in the past is much less obvious. If one looks at a present day world map it is tempting to assume that subduction zones either form at continental margins, to produce an Andean-type situation, or at some distance from the margin, and presumably within oceanic crust formed by spreading, to form the island arcs and marginal basins of the western Pacific. In this latter case crust older than the arc itself

would be isolated behind it. This is one possible mode of origin for the West Philippine Sea, which is discussed on page 458 of this issue. If, however, a subduction zone forms at some distance from a continental margin and dips away from it, the zone will ultimately collide with the margin, as a result of the destruction or resorption of the intervening oceanic crust, and the polarity of the subduction zone is presumably reversed to produce, finally, an Andean-type margin. This process has sometimes been referred to as 'Benioff-plane reversal'. Conversely a subduction zone which forms at or near to a continental margin, and dips beneath it, may subsequently be rafted away from it by a process of 'back-arc spreading'; that is, the formation of a small ocean basin behind the arc by a process analogous, perhaps equivalent, to spreading about a mid-ocean ridge crest. Clearly such a process would mean that the back-arc basin would be floored by crust which is younger than the arc itself.

The first person to suggest the process of 'back-arc spreading' was Karig (*J. geophys. Res.*, **75**, 239; 1970) and he documented it primarily in terms of the sediment thickness and near-surface structure revealed by seismic reflection profiling. He envisaged it as an episodic process with phases of spreading occurring immediately behind the arc and producing basins separated by ridges of former island-arc material and younging towards the arc. This then was Karig's model for the origin of the western Pacific basins, including the Philippine Sea, the largest of the back-arc basins. It is only fair to say that much of the geophysical and geological data (which includes deep-sea drilling results) obtained since the time of Karig's initial suggestion have tended to corroborate his ideas, and his model is now widely accepted.

Precisely a year ago, however, in this journal (*Nature*, **240**, 453; 1972) Ben-Avraham *et al.* made a case for the existence of an extinct spreading centre in the West Philippine Sea, the spreading axis of which trends NW-SE and the age of which probably exceeds that of the Mariana island arc to the east. They felt therefore that it was more compatible with formation in the main Pacific basin prior to the formation of the arc than with the concept of back-arc spreading. In this issue Karig *et al.* present a rejoinder armed with new data acquired as a

result of Deep-Sea Drilling Project leg 31 in the Philippine Sea. They maintain that it was quite fortuitous that the single bathymetric and gravity profile across the 'Philippine Ridge', of Ben-Avraham *et al.*, seems to be consistent with the model of an extinct spreading centre. Further work, they claim, favours the original interpretation of the feature by Hess (*Bull. Geol. Soc. Amer.*, **59**, 417; 1948) as a strike-slip fault. Although the interpretation of Ben-Avraham *et al.* was in many ways convincing, its greatest weakness was the paucity of data on which it was based, essentially little more than one profile. Also the relief of the central rift area is somewhat excessive and more reminiscent of present day transform faults than ridge crests. The symmetrical magnetic anomalies were the most convincing evidence put forward, but a worrying aspect of these was that they were interpreted as reflecting reversals during late Mesozoic time when from other areas and palaeomagnetic studies

there are strong indications that the Earth's magnetic field was of a single, normal, polarity for much of the time.

In spite of the fact that Karig *et al.* feel that their new data invalidate the interpretation of the central basin feature as a ridge rather than a fault, they admit that they are unable as yet to provide an unequivocal answer to the origin of the West Philippine Basin. Many questions remain unanswered not least of which is the extent and role of Tertiary volcanism which significantly post-dates the origin of the crust, and the occurrence of non-calcareous basal sediments in some areas which on the face of it argues against formation at an elevated spreading centre. It is clear that the nature and origin of marginal seas is going to be a battlefield for earth scientists for some time to come; a battlefield which may ultimately become a legal and political one in view of the potential economic importance of marginal basin geology.

From a Correspondent

RIBOSOMAL RNA

Mechanism of Amplification

from a Correspondent

RIBOSOMAL gene amplification during oogenesis in *Xenopus* (Gall, *Proc. natn. Acad. Sci. U.S.A.*, **60**, 553; 1967) suggested the possibility that a similar mechanism of specific gene amplification acts during the differentiation of cells to produce large quantities of a single or a few types of protein. In this way anything learnt about the mechanism of rDNA amplification might have a wider and more important impact on developmental biology. Two recent tests of this idea, however, one for silk fibroin synthesis in the silk gland of the moth *Cecropia* (Suzuki, Gage and Brown, *J. molec. Biol.*, **70**, 639; 1972) and one for globin synthesis in differentiating blood cells (Bishop, Pemberton and Baglioni, *Nature new Biol.*, **235**, 231; 1972), have shown that specific gene amplification during differentiation is not a general phenomenon.

In spite of this apparent relegation of ribosomal gene amplification to the level of biological oddity, it has continued to excite interest on four counts. Intriguing and novel molecular mechanisms may be involved (see Tocchini-Valentini and Crippa, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2769; 1971); it may be related to important features of the maintenance and evolution of multiple gene loci (Buongiorno-Nardelli, Amaldi and Lava-Sanchez, *Nature*, **238**, 134; 1972;

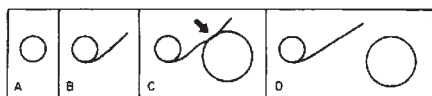
Amaldi *et al.*, *Nature*, **242**, 615; 1973); the nature of dosage compensation both in rRNA synthesis and rDNA amplification observed in heterozygote (+/nu-) animals (Macgregor, *Nature new Biol.*, **246**, 81; 1973) and of the 'dominance' phenomena found in interspecies crosses (*Nature*, **246**, 60; 1973) may tell something of the mechanisms controlling rRNA synthesis and rDNA amplification.

Rolling Circle Intermediate

Recent work by Hourcade, Dressler and Wolfson (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2926; 1973) provides convincing evidence that rDNA amplification proceeds by way of a rolling circle intermediate. What they have done is to isolate by CsCl density gradient centrifugation rDNA from amplifying ovaries, taking great care to avoid shearing forces which might cause breakage of DNA molecules. They looked at this DNA with an electron microscope and found that although most molecules are linear, 2-5% are circles—and rolling circles (circles with tails) which range in size from $8-140 \times 10^6$ daltons. By measuring contour lengths they were able to show that most of the circles with a mass of less than 40×10^6 daltons fall into discrete size classes corres-

ponding to multiples of the basic repeating unit (mass 8×10^6 daltons) found in rDNA. They suggest that the reason why larger circles do not is that the error in measurement causes overlap of neighbouring size classes.

Circles outnumber rolling circles by six to one, but rolling circles also fall into the same multiple series of the basic ribosomal gene. It might be argued that the tail on these rolling circles was originally inserted on the circle at both ends to give a θ -shaped Cairns type replicative form and that one end has been liberated by breakage during isolation. Breakage of a Cairns form would always give tails shorter than the circles and presumably also circles with two tails; in fact 85% of tails are longer than the circle to which they are attached and moreover no circles with two tails were observed.



Hourcade *et al.*'s idea of how a rolling circle intermediate is expected to produce, by a recombination event in its tail, multimer-length amplified DNA circles. This recombination event could also occur if the tail has been detached from the rolling circle and exists as an intracellular concatemer. Single line represents a DNA double helix.

Hourcade, Dressler and Wolfson also saw two other DNA forms, a Y-shaped structure with three unequal arms which may be produced by breakage within a rolling circle, and more important, two θ -shaped Cairns forms, which may indicate that both rolling circle and double rolling circle or Cairns type replication proceed together or that one precedes the other during amplification.

Since rolling circles represent such a small proportion of all molecules, it may be that they represent a minor non-rDNA component with the same buoyant density and same basic repeat length as rDNA. Hourcade *et al.* used the technique, originally applied by Brown, Wensink and Jordan (*J. molec. Biol.*, **63**, 57; 1972), of partial denaturation of DNA by heat, alkali or formamide, followed by electron microscopy to map denatured and native regions. As expected they found that the same denaturation pattern exists in linear molecules, circles and rolling circles proving their sequence identity.

Similar work by Bird, Rochaix and Bakken (*Molecular Cytogenetics*, Oak Ridge Symposium, 1973) confirms the presence of rolling circles in rDNA from amplifying ovaries. They have also used tritiated thymidine to label replicating DNA followed by autoradiography in the electron microscope which allows them to select rapidly for replicating molecules (those which have

silver grains over them). It turns out that grains are seen over rolling circles, providing evidence that the rolling circles observed really are synthesising DNA.

Other Mechanisms

Other molecular mechanisms for rDNA amplification have been proposed. Buongiorno-Nardelli *et al.*, from a study of a large number of amphibian species, provide evidence for a $2n$ relationship between the number of ribosomal genes per diploid cell (R) in different species, and also that a similar $2n$ series exists for the total number of amplified oocyte nucleoli (A) in these same species. They found three cases in which $R=A$ while there were nineteen cases in which $R>A$ and none where $R<A$. To account for these observations they proposed that amplification proceeds by the following mechanism; an initial series of crossover events release each rRNA gene as a monogenic circle, there are thus R circles, these circles replicate by the double rolling circle mechanism, characteristic of the replication of λ phage in *Escherichia coli*, giving rise after n rounds of replication to circles with $2n$ genes. Thus R is equal to or greater than A , depending on whether all the genes are liberated or whether they are liberated as multiples of the repeat unit, but R is never less than A . The single rolling circles observed by Hourcade *et al.* and, more important, the arithmetic rather than geometric increase in the size of circles, argues specifically against the Buongiorno-Nardelli model for amplification although not against their general conclusions about its possible significance as a correction mechanism for multiple gene loci.

Tocchini-Valentini and Crippa (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2769; 1971) suggested that rDNA amplification might proceed by way of the synthesis of DNA by a reverse transcriptase on an initial complete RNA transcript of the ribosomal gene repeat unit. They provided evidence for the presence in amplifying ovaries of *Xenopus* of such an RNA transcript which is much larger (47S) than the normal rRNA (40S) precursor and also contains sequences complementary to rDNA. The presence of an RNase resistant RNA-DNA complex containing high molecular weight RNA was predicted and found. A rifampicin derivative, which inhibits viral reverse transcriptase, was also found to inhibit rDNA amplification but not main bond DNA synthesis. These observations taken together seemed convincing enough; however, Bird, Rogers and Birnstiel (*Nature*, **242**, 226; 1973), using techniques of greater refinement and sophistication, were unable to verify any single one of them and concluded that reverse transcription

is an unlikely solution to the problem of the amplification mechanism. The rolling circle DNA forms seen by Hourcade *et al.* and Bird *et al.* add further weight to the evidence against reverse transcription as the major molecular mechanism for rDNA amplification but do not exclude its operation early in amplification or at a low level throughout amplification.

Origin of rDNA Circle

Several questions remain unexplained by existing work, the main one being the origin of the initial rDNA circle, which may formally be derived in four ways. One is by maternal inheritance as an episome (Wallace, Murray and Langridge, *Nature new Biol.*, **230**, 201; 1971) and has been disproved by the demonstration of the lack of maternal inheritance for rDNA amplification in *Xenopus laevis-mulleri* hybrids (Brown and Blackler, *J. molec. Biol.*, **63**, 75; 1972). Second, an RNA transcript may give rise by reverse transcription to a DNA molecule which is circularised by a DNA ligase and then replicates by the rolling circle, double rolling circle or Cairns type intermediates. The attraction of this model is that it eliminates the need to postulate production of rDNA circles by crossing over within the gene cluster. Third, crossing over within the rDNA cluster might generate all sizes of circles from single genes to the complete locus. The fourth possibility is semiconservative DNA replication of a part or the whole of the rDNA locus following by excision at growing points and circularisation with ligase or crossing over. A corollary of this question is whether representatives of all chromosomal rRNA gene sequences are present in amplified rDNA.

HORMONES

Binding to Receptor

from a Correspondent

STUDIES with rat ventral prostate have shown that androgen-sensitive tissues contain receptor proteins which interact with the androgen, and in the case of the naturally occurring hormone testosterone, its reduction to 5α -dihydrotestosterone is an initial and essential step in the interaction. Knowledge of the extent to which 5α -reductase and the receptor protein occur in tissues and of the ability of other potent steroidal androgens, which are not converted to 5α -dihydrosteroids, to interact with the receptor is limited. These aspects have been investigated in two recent contributions.

One might expect a close functional relationship between the receptor and enzyme proteins. Mainwaring and Mangan (*J. Endocr.*, **59**, 121; 1973)

measured the concentration of receptor protein and 5α -reductase activity in the accessory sex glands from four species, rat, mouse, guinea pig and rabbit, and also in a number of other tissues which might be expected to contain the receptor, for example, muscle, because of the anabolic effect of the androgens, kidney tissue and mouse tumours which require androgens for growth. Binding activity was present in rat and mouse testes, mouse kidney, although this tissue seemed to take up testosterone as well as 5α -dihydrotestosterone, and significant nuclear binding of radioactivity was demonstrated in androgen-dependent but not in androgen-independent tumours in mice. With the exception of the levator ani muscle of the guinea pig, the binding and reductase activity in other muscles from the four species were small.

Although high activities of the 5α -reductase were found in the prostate and epididymis of the rat and mouse with smaller activities in seminal vesicles and kidneys, the testes and muscles investigated contained negligible activity. Surprisingly, rabbit prostate contained only about half the amount of receptor protein as rat prostate and 5α -reductase activity was virtually absent. These findings therefore show considerable variability in androgen-sensitive tissues from various species both in their concentration of receptor protein and their ability to convert testosterone to 5α -dihydrotestosterone.

Liao *et al.* (*J. biol. Chem.*, **248**, 6154; 1973) studied the ability of natural and synthetic androgens to compete with 5α -dihydrotestosterone for binding to the cytoplasmic receptor protein and for retention by prostatic cell nuclei. There was a good correlation between the relative androgenicity of the synthetic steroids and their ability to compete with 5α -dihydrotestosterone for binding to the receptor protein and for retention by prostatic nuclei. Testosterone only competed slightly with 5α -dihydrotestosterone at the cytoplasmic level, however, although it did reduce retention of 5α -dihydrotestosterone at the nuclear level. Unlike testosterone which depends on reduction by 5α -reductase for its action, many synthetic androgens seem to bind directly to the receptor protein, without being reduced by 5α -reductase. The 17β -hydroxy group was essential for binding to the receptor protein and neither 17α -oxosteroids nor 17α -hydroxysteroids showed any significant binding.

From this study of a large number of steroids the authors conclude that the flatness of the steroid molecule around ring A is a determinant of the firmness of androgen binding to the receptor and that steric factors are more important in binding than the detailed electronic structure.

X-RAY SOURCES

Luminosity Function

from a Correspondent

RECENT investigations of the luminosity function and spatial distribution of the galactic X-ray sources are bringing astronomers nearer to an understanding of their origin. The distribution of a class of sources within our Galaxy can indicate an association with a known stellar population and hence give an idea of how they fit in with present ideas about stellar evolution. Dilworth, Maraschi and Reina (*Astron. Astrophys.*, **28**, 71; 1973) and Margon and Ostriker (*Astrophys. J.*, **186**, 91; 1973) conclude, in accord with earlier suggestions, that the galactic X-ray sources fall into at least two, and possibly three, distinct groups.

First, there are the sources populating the whole disk of the galaxy. These consist of two distinct types. There are the 'steady' sources, which have a wide spread of luminosities and among which a number have been identified with the remnants of supernovae; there are also

the 'highly variable' sources whose luminosities are all fairly close to 1.3×10^{37} ergs s^{-1} . The sources in this second subgroup are probably almost all associated with binary systems in which X-ray emission arises from accretion onto a compact object. Unresolved sources from this population could account for about half the galactic background emission.

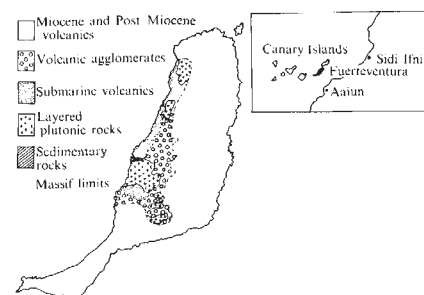
Second, there is a population of sources at the centre of the Galaxy. This population seems to consist of a single class of variable, high luminosity sources, with a relatively small spread in luminosity around 10^{38} ergs s^{-1} . This luminosity corresponds to the theoretical (Eddington) limit for the luminosity of an object with a mass about equal to that of the Sun. If the luminosity of such an object were much more than this, radiation pressure would exceed gravity in the surface layers and mass loss would ensue. This is not very compatible with the idea that accretion of matter is the process which powers the X-ray source. Thus it is comforting that on average the Eddington limit is not exceeded, and, looking ahead,

Uphrust Ocean Floor in the Canary Islands?

THE Betancuria massif, which forms the western part of the Island of Fuerteventura in the Canary Islands, has a core of layered gabbros, pyroxenites and peridotites associated with pillow lavas, siliceous and calcareous sediments and volcanic agglomerates. The whole is cut by a dyke swarm which is especially dense at the contact between the layered plutonic rocks and the other components of the formation, and was subsequently overflooded by three different basalt series ranging from Miocene to prehistoric. Finally, a carbonatite complex and syenitic and trachytic ring dykes and plugs were intruded.

Even on the basis of this superficial geological description there is a clear resemblance between the Betancuria massif and other ophiolite complexes such as the Troodos massif of Cyprus; and in *Nature Physical Science* last Monday (December 17) Gastesi proposes that Betancuria, like Troodos, is an uplifted segment of ancient oceanic crust (see figure). Of course, Gastesi's analysis is based on a much more detailed review of the geology than that just given; but the more rigorous examination strengthens the conclusion that all the units of the Betancuria massif are consistent with the ocean floor interpretation, and not just the plutonic rocks as proposed by Fúster *et al.* (Instituto Lucas Mallada, CSIC, Madrid, 1968). The principal differences between Betancuria and Troodos are chemical, the Betancuria rocks

being generally the more alkaline; but these do not appear to be serious enough to invalidate the basic conclusion.



Age data from the Betancuria massif are few and fail to give anything like a complete picture, although recent radiometric ages for the dykes lie in the range 46–28 million years. For the time being, therefore, Gastesi feels that two rather different patterns of evolution are possible. In the first, Betancuria was formed as part of the mid-Atlantic ridge, the radiometric ages representing "a more recent metamorphic episode in the development of the Atlantic margin". The second possibility is that the massif formed away from the mid-Atlantic ridge during the mid-Tertiary, perhaps as part of an abortive attempt to start a new ridge. Either way, what is now required is detailed geophysical work to discover how closely the structure and physical properties of Betancuria resemble those of the modern ocean floor.

Margon and Ostriker suggest that because of the small spread in luminosity about this limit, similar groups of X-ray sources in other galaxies could be used as 'standard candles', much in the same way as Cepheids have served till now.

Third, Dilworth, Maraschi and Reina argue that present observations do not rule out the possibility of a third class of low luminosity sources ($\sim 10^{32}$ ergs s^{-1}) which are at present indistinguishable from the isotropically distributed sources which are thought to be mostly extragalactic. This class cannot account for more than about 10% of these but could give rise to the rest of the galactic background emission.

PALAEOMAGNETISM

Past Secular Variation

from our Geomagnetism Correspondent

HISTORICALLY, palaeomagnetic data have been seen largely as indicators of the Earth's ancient dipole, rather than non-dipole, field. This is so whether they refer to the comparatively recent past, in which case the dipole orientation is given directly in terms of the Earth's geographic or geomagnetic coordinates, or to the more distant past, in which case the frame of reference is changed by continental drift and the dipole hypothesis appears as an assumption rather than as something to be determined directly from the data. In either case the scatter in measured palaeomagnetic directions arising from the effects of the much more rapidly varying non-dipole field is usually seen as an embarrassment, and is 'averaged out' mathematically from the results of a large number of rock samples.

But in recent years there has been a growing interest in the scatter itself, or rather in what useful information may be derived from what has traditionally been regarded as a nuisance. If the ancient non-dipole field introduces scatter or 'noise' into the palaeomagnetic dipole 'signal', the argument goes, it should be possible to work back from the scatter to determine the old non-dipole field. Unfortunately, the idea is more easily stated than put into practice because not all of the observed scatter is produced by the non-dipole field. Experimental error, local magnetic anomalies and dipole wobble may increase the scatter and incomplete sampling may either underestimate or overestimate it; and all these and other factors must be controlled or measured in order to assess what part of the scatter is truly due to the phenomenon under investigation.

Some of the difficulties involved are well illustrated in an attempt by Watkins (*J. geophys. Res.*, **78**, 7763; 1973) to determine the secular variation during

the Brunhes epoch using lava flows from Réunion Island in the Indian Ocean region. This is not the first time that Réunion rocks have been used for this purpose. A few years ago, Chamalaun (*J. geophys. Res.*, **73**, 4647; 1968) measured the palaeomagnetic directions of forty-nine Réunion lava flows, obtaining what Watkins *et al.* (*Geophys. J.*, **28**, 1; 1972) later calculated to be an angular standard deviation of about 20° in the virtual geomagnetic poles (VGPs) with upper and lower confidence limits of 23.2° and 17.5° , respectively. But Chamalaun used an average of less than two samples per lava flow, and so his data could not be used to calculate, and hence remove the effects of, the within-lava variation. Cox (*Earth planet. Sci. Lett.*, **6**, 257; 1969) thus rejected the final result on the grounds that the sampling was inadequate to allow a true determination of the ancient secular variation; and the field was left open to a much more thorough investigation involving more intensive sampling.

By sampling at a level of at least six samples per lava flow, Watkins clearly hoped to be able to obtain a result which would overcome the objections of Cox and thus be accepted as a true estimate of the ancient secular variation. Accordingly he carefully collected and measured 160 cores which were restricted to the Brunhes epoch by the use of previous potassium-argon ages, field polarity measurements and known stratigraphy, and which were restricted to locations at which accurate orientation could be carried out with respect to known geographic bearings. For flows that did not have anomalous palaeomagnetic directions (three did), the angular standard deviation of the VGPs arising solely from secular variation (that is, after removing other contributions to scatter such as that due to within-flow variation) was 17.7° with upper and lower confidence limits of 22.8° and 14.4° , respectively—which is little lower than the standard deviation obtained by Chamalaun using less than two samples per flow and thus still much higher than that to be expected on the basis of the present field.

Taken at face value the new result seems valid enough. The range of known radiometric ages involved, for example, is about 0.4 million years or over half the length of the Brunhes epoch, suggesting that an adequate range of time has been sampled. On the other hand, if the Watkins result really is valid, its similarity to the Chamalaun result would imply that there is little to be gained by increasing the level of sampling to over six samples per flow. Although this is not impossible, it is intuitively unlikely and conflicts with some previous experience.

But what really throws doubt on the new result is the fact that the angular

SOLAR SYSTEM

Vulcan?

from a Correspondent

INSTRUMENTS aboard Skylab may detect an object moving in an orbit closer to the Sun than the planet Mercury, according to Henry Courten of Dowling College at Oakdale, New York. Courten has photographed the regions around the Sun regularly at total eclipses since 1966. During the 1970 eclipse in North America, one object was photographed near the Sun from three widely spaced observing sites. These images could be of a previously unknown member of the Solar System orbiting close to the Sun.

Courten believes a planetesimal "between 80 and 500 miles in diameter" moves in an orbit about 0.1 AU from the Sun. On his photographs, this appears as a star-like object between magnitude +7 and +9. Other images on Courten's plates indicate that a complete asteroid belt may exist between Mercury and the Sun.

Courten is a guest investigator on the Skylab white-light coronagraph experiment. "If intra-Mercurial, solar-orbiting objects exist, they will eventually pass through the coronagraph field of view", he says. The only problem is that the sensitivity of the Skylab instrument borders on the limit of brightness of Courten's photographic images.

dispersion supposedly due solely to ancient secular variation is significantly higher than that due to the present field, and is, moreover, out of line with ancient dispersion results from other Indian Ocean islands such as the Comores Islands, the Crozet Islands and Amsterdam Island. This could, of course, be due to a regional secular variation anomaly which affects Réunion but not the other islands. Watkins believes it more likely, however, that an insufficient number of separate lava flows have been sampled, notwithstanding the long time range. Although he took over six samples from each flow, the total number of flows involved was eighteen compared with Chamalaun's forty-nine. Normally, eighteen intensively sampled flows might be expected to yield a valid result where forty-nine poorly sampled flows might not; and indeed Watkins's new value may turn out to be correct. Unfortunately, the level of doubt is sufficient to require yet another investigation involving not only high density sampling but a much larger number of flows.

ZOOLOGY

Pogonophoran Phylogeny

from a Correspondent

THE first international symposium on Pogonophora, which took place in the University of Copenhagen on November 1-3, had as its main topic the evolution and phylogeny of the group, but the discussions covered all aspects of the biology of these enigmatic deep-water benthic invertebrates. The symposium was organised by A. Nørrevang, with financial assistance from the Danish Research Council.

On the first day there were informal discussions and demonstrations of material, including some live animals brought along from the Norwegian fjords by T. Brattegaard (University of Bergen Biological Station). The second day, under the chairmanship of J. B. Kirkegaard (University of Copenhagen), started with some very intriguing ideas of his colleague H. Lemche on the embryology and phylogeny of the main

invertebrate groups. E. B. Cutler (University of Syracuse, Utica) then reviewed the occurrence of supposed protostomian and deuterostomian characters in Pogonophora and suggested that the group should be regarded as related to forms ancestral to both of these major subdivisions of the Eumetazoa. J. van der Land (University of Leiden) and A. Nørrevang reported on the morphology of a newly discovered Atlantic species of *Lamellibrachia* and suggested that this genus might be regarded as a subgroup of the Annelida. M. Webb (University of Durban, Westville) provided a thorough account of the excretory and genital systems of the Pacific species of *Lamellibrachia*, which he preferred to continue to regard as aberrant pogonophores. T. Bakke (University of Bergen Biological Station) briefly described the important early stages in the development of the artificially fertilised egg of *Siboglinum*; the cleavage could not be regarded as either spiral or radial.

At this stage in the session R. Siewing (University of Kiel) read a translation of a letter from A. V. Ivanov (Zoological Institute, USSR Academy of Sciences, Leningrad) regretting that he was prevented from attending, and announcing the discovery of some new facts on gastrulation in *Siboglinum*. E. Southward (Marine Biological Association, Plymouth) discussed the fine structure of the recently-discovered segmented and setate hind-end of *Siboglinum*, and traced its origin from the posterior segment of the larva, comparing the number of annelid characters and more general characters thus revealed. B. L. Gupta (University of Cambridge) and C. Little (University of Bristol) provided a far reaching and comprehensive account of many aspects of the fine structure of Pogonophora in comparison with other invertebrates, and pointed out the pitfalls awaiting the investigator trying to deduce relationships from functional morphology: Gupta finally expressed a doubt that pogonophores are coelomate animals at all. A. J. Southward (Marine Biological Association, Plymouth) summarised what had been found out or deduced about the feeding of these free-living yet gutless animals, and stressed the phylogenetic problems involved in the evolution of an epidermal mechanism of food absorption; he wondered what environmental conditions and selective pressure could lead to the loss of an existing internal digestive system.

Brattegaard then analysed the fauna associated with pogonophores, especially in the fjords; there seemed to be no consistent relationship between a pogonophore species and a single benthic community, though the organic content of the habitat seemed to be important as were abiotic factors such as temperature and particle size of the sediment.

The third day of the meeting, under the chairmanship of C. Nielsen (Marine Biological Laboratory, Elsinore), opened with some ideas of Siewing on phylogeny in relation to embryology and development of the coelomic cavity: the Pogonophora were placed well down the phylogenetic tree, close to the origin of the archicoelomates (Echinodermata and Lophophoria), with the Spiralia (for example, Annelida, Arthropoda) regarded as an offshoot of the main line of development.

At the final dinner, held at the Zoological Museum, Nørrevang was able to read the full abstract of Ivanov's intended paper, just delivered by express post. This confirmed a reassessment of the embryology of the group, no longer regarded as having radial cleavage, and it was suggested that Pogonophora form a separate group of the Eumetazoa, distinct from the Protostomia and Deuterostomia.

INSECTICIDES

Knockdown and Kill

from a Correspondent

NATURAL pyrethrins have been used extensively as insecticides partly because of their toxic properties but principally because of their rapidity of action—effects are observable in minutes against hours for chlorinated hydrocarbons. Doses applied topically cause uncoordination leading to knockdown of flies, mosquitoes and cockroaches. High doses cause death but at lower levels there may be recovery although this is usually prevented by the addition of a synergist such as piperonyl butoxide.

The advent of synthetic pyrethroids has stimulated further study into structure/activity relationships as these substances can occur as optical and geometrical isomers. 'Knockdown' was the subject of a symposium organised by the Biophysical and Physiochemical Panel of the Pesticides Group, Society of Chemical Industry, held on November 23.

P. E. Burt (Rothamsted Experimental Station) discussed the possibility of knockdown and kill being associated with different sites of action; in his view both actions are on the central nervous system and the peripheral nerves are not involved. This view was also taken by M. G. Ford (Portsmouth College of Technology) from a study of doses applied topically on the desert locust. A logarithmic plot of the time of 50% response against weight of applied dose gave linear relationships for kill and knockdown using the synthetic pyrethroids bioallethrin and bioresmethrin.

M. Elliot (Rothamsted Experimental

Station) discussed various synthetic pyrethroids in relation to knockdown and kill. Compounds with good knockdown are usually relatively poor as killing agents but their rapid action is probably due to a higher polarity which facilitates rapid penetration of the insect cuticle. Knockdown can be correlated with polarity, which is related to R_F values measured in appropriate chromatography systems.

J. Lhoste (Procida, Paris) related speed of knockdown of mosquitoes (*Aedes aegypti*) with the stereochemical structure of eight allethrin isomers. Test compounds were applied to a flat 'mosquito' coil of suitable composition, which burns slowly volatilising a substantial proportion of the insecticide. The most active isomer had the same stereochemical configuration as natural pyrethrins. J. C. Wickham (Wellcome Research Laboratories, Berkhamsted) emphasised the need for biological tests in the most realistic conditions possible in the laboratory and particular attention must be given to the size of aerosol droplets, the size of the test chamber and the adjuvant solvents in test against houseflies, mosquitoes and cockroaches; this supplemented Burt's earlier observation that there is a marked difference in effect on applying pyrethrin I in acetone to the ventral and dorsal surfaces of the housefly but no difference when the solvent is dodecane.

Moving away from pyrethroids, N. R. McFarlane (Shell Research) recorded studies on houseflies giving time and dose response curves of a closely related series of oxime carbamates. Using an analogue computer it is possible to construct models relating results to factors such as partition.

Planetary Alignments, Solar Activity and Climatic Change

JOHN GRIBBIN

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Historical data suggest a relation between the changing level of solar activity and climatic change on Earth. Since there is also evidence that the solar cycle of activity is influenced by the alignments of the planets, through tidal interactions with the Sun, it seems that climatic change might be predicted by studying these alignments.

THERE may be a causal chain linking changes in the alignments of the planets, through their tidal effect on the activity of the Sun, with climatic change on Earth. Although the separate components of this chain have been discussed before, it seems that they have not previously been linked in this way to form a coherent whole. If these ideas are correct, they provide the best basis yet found for predicting climatic changes in the immediate future.

It is convenient to assess the evidence for such a chain in the reverse direction—that is, starting from the Earth. There is good evidence that the rate at which the terrestrial length of day changes is influenced by the mean level of solar activity (see, for example, ref. 1), and although the question of a direct link between specific events on the Sun and specific changes in the length of day² seems more contentious³ it is clearly relevant to consider by what mechanism the longer term variations in solar activity affect such a gross property as the spin of the Earth. Since large scale movements of air masses associated with seasonal changes in the atmosphere are already known to produce a component in the variation of the length of day with a period of ~ 1 yr, it seems reasonable to look for the atmospheric link between changes in the activity of the Sun and changes in the spin of the Earth.

Solar Storms and Weather

In spite of the difficulty of establishing that individual large solar storms produce detectable discontinuous changes in the Earth's spin rate, there is little doubt that an increase in the level of solar cosmic rays arriving at the Earth can cause a short term change in the weather at high latitudes. Roberts and colleagues have shown (see ref. 4 and references cited therein) that troughs at high latitudes are amplified when the level of cosmic rays reaching the ionosphere (revealed, for example, by auroral activity) increases abruptly.

This discovery has obvious implications for improving short term local weather forecasting in some parts of the world. But as far as climatic changes are concerned it is

necessary to look at data averaged over at least one cycle of solar activity. King (personal communication and ref. 5) has pointed out that "it is now well established that the density and temperature of the atmosphere above about 100 km vary markedly with solar activity during . . . the 11-yr solar cycles", and he has shown that there is a positive correlation between solar radiation changes which occur during the solar cycle and meteorological variations. For example, as the level of solar activity, revealed by the number of sunspots, decreases, there is a decrease in mean temperatures and an increase in winter rainfall at Beirut. These changes seem to be related to changes in the altitude of the 500 mbar surface⁶. But even observing variations over an 11-yr period—or over several solar cycles—is not sufficient to determine patterns of climatic change.

Historical Data

Fortunately, there is a great deal of meteorological data available, and records of "good" and "bad" seasons go back for almost a thousand years⁷, in England at least. Lamb has used some of these data to determine variations of climate in the northern hemisphere and he explains them in terms of successive movements of climatic zones to south and north over periods of hundreds of years^{7,8}. According to Winstanley⁹, 1930 marked the peak of a northward excursion of climatic zones, and we are now experiencing a southward shift which has, among other effects, caused the serious drought in Africa just south of the Sahara in recent years. He foresees continued southward movement of both the Sahara and the Rajasthan Deserts until at least 2030 assuming that the present variation is part of a 200-yr cycle. The evidence for a cycle of approximately that period comes both from climatic records⁷ and from estimates of variations in the radiocarbon content of the atmosphere¹⁰; the cycle last peaked, before 1930, in the early part of the eighteenth century. It is also possible that 1930 marked the "peak" of a 700-yr cycle, although the data are not sufficient to show conclusively that such a cycle really exists.

It seems obvious to ask if there is any link between these longer term effects and the mean level of solar activity. The changes in atmospheric radiocarbon levels are particularly tantalising, since they hint at changes in the mean cosmic ray flux with period of approximately 200 yr; unfortunately, however, the only record of solar activity available which goes back for more than a few years is sunspot number, and as King points out⁵ that is a relatively crude index. Nevertheless, when sunspot numbers are plotted for this century (Fig. 1) it is immediately striking, in the present context, that the maximum number of spots, at the time of maximum solar activity, was itself a minimum in the cycle which peaked just before 1930, at the "peak" of the 200-yr climatic cycle mentioned by King. But good

data for the numbers of sunspots at the peaks of sunspot cycles go back only to 1820—fourteen complete cycles—and there are no numerical data for cycles before 1750. Similarly, climatic data become more qualitative as one searches further into the past, and detailed quantitative data are only available for years since the mid-nineteenth century.

Possible Implications for Prediction

Attempting to establish a correlation between the two sets of data might thus seem to be foolhardy, and indeed although such attempts have been made they have met

there is a close link between the height of the tide raised on the Sun by various planetary alignments and the dates of sunspot maxima and minima; there is also a weaker link between the variation of tidal height and sunspot number¹³. That makes it possible to predict future solar activity levels, and the dates of maxima and minima, rather more accurately than simply extrapolating the average 11.1-yr cycle. And if the level of solar activity affects climate, as is strongly suggested by King's work⁵, that in turn provides a means for predicting climatic change. Perhaps the similarity between the approximately 200-yr variation of climate and atmospheric radiocarbon levels mentioned by Winstanley⁹ and the 170 to 180 (or 179?) year variation in solar activity¹³ is more than a coincidence.

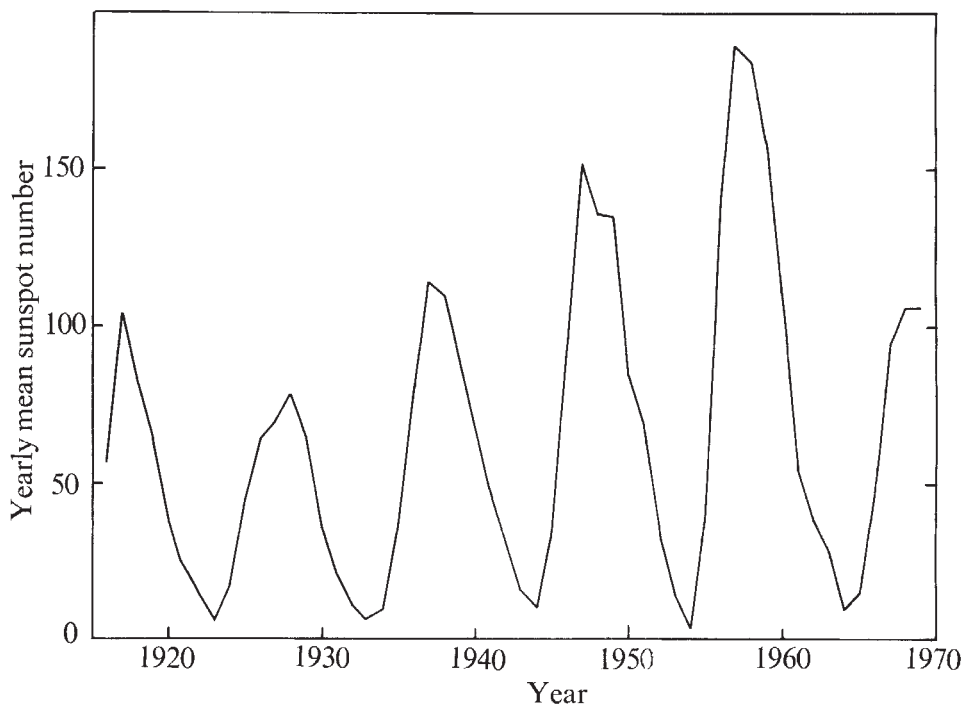


Fig. 1 Yearly mean sunspot numbers during this century. (After King⁵.)

with some opposition. But the urgency of climatic research has been highlighted by recent events in Africa and India, and although it would still perhaps be foolhardy to make any conclusions drawn from comparison of these data sets the basis for immediate action, it does seem to be worthwhile to present these conclusions for discussion at the present time, when there is no completely satisfactory theory to explain climatic changes. Lamb (ref. 8, p. 443) has discussed previous attempts to relate climatic changes to long solar cycles. One of the most important of these is the discovery of a correlation between climatic series, solar variation and radiocarbon levels over more than 2,000 yr (see refs 11, 12); but in addition to the difficulty of relating past climatic changes to past changes in solar activity there is the pressing problem of predicting future climatic trends. Assuming that the correlations outlined by Lamb and discussed in detail in the references he cites do have a secure basis, is there any hope of predicting future solar activity and thus estimating future climatic effects?

Wood¹³ has noted that the maximum smoothed monthly number of sunspots for each solar cycle seems to repeat after about 170 to 180 yr. He made this discovery when analysing the available sunspot data for evidence of an influence which might be attributed to the tidal effects of the planets on the Sun; I have mentioned elsewhere¹⁴ that if this is a periodic effect then it might be linked to the 179-yr period between the alignments of the outer planets. Certainly, Wood has produced convincing evidence that

Further speculation along these lines would be inappropriate here, even granted the need for investigating all possibilities when concerned with predicting climatic change. But it does seem that climatologists and others concerned by large scale effects of changes in the atmospheric circulation such as those reported by Winstanley^{6,9} could do worse than to examine the predictions of solar activity made by Wood¹³ on the basis of planetary alignments.

I am grateful to a referee for comments.

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mRNA Transcription linked to the Morphological and Plasma Membrane Changes induced by Cyclic AMP in Tumour Cells

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Studies on a rat sarcoma link plasma membrane alterations to the activity of DNA-directed RNA polymerase II.

THE ubiquitous cell regulatory role of adenosine-3':5'-cyclic monophosphate (cyclic AMP) on a multiplicity of metabolic processes is mediated through modulation of cyclic AMP levels by intracellular adenyl cyclase and cyclic AMP-phosphodiesterase activities¹. Although the 'second messenger' hypothesis² has clearly defined a major role for cyclic AMP in hormonally mediated responses, reports³⁻⁵ on the establishment of density dependent inhibition of growth in non-contact inhibited cells as well as differentiated functions⁶⁻⁸ by cyclic AMP and its dibutyl derivative suggest another major role for cyclic AMP in cell division and neoplasia. Specifically, the intracellular concentration of cyclic AMP has been implicated in the regulation of cell division *in vitro*. Rapidly growing cells exhibit relatively low levels of cyclic AMP while increasing concentrations are associated with a progressive increase in cell division times⁹. When cyclic AMP or metabolically active derivatives are incorporated in the growth media of either carcinoma⁶⁻⁸ or sarcoma³⁻⁵ cells in continuous culture, there is not only a marked growth inhibition but also a dramatic alteration in cell morphology towards that of the normal differentiated cell. Apparently related to the morphology and growth inhibition are changes in the cell surface^{10,11}. This communication links these cyclic AMP-mediated effects to the induction of mRNA by activation of nuclear DNA-directed RNA polymerase.

The *in vitro* XC rat sarcoma continuous cell line was selected as a model system for study because of its rapid growth in culture, low intracellular concentrations of cyclic AMP, and its ability to form giant syncytial cells when co-cultivated with cells producing murine leukaemia virus (MuLV)¹². Electron microscopy has revealed MuLV envelope fusion with the XC cell membrane¹³ and a heat-labile lipoprotein from MuLV has been implicated as a fusion-inducing factor¹⁴. Thus syncytial cell formation seems to be a membrane phenomenon analogous to the formation of heterokaryons by Sendai virus. We have previously demonstrated that cyclic AMP incorporated in the growth media of XC cells blocks their ability to form syncytia, decreases their agglutinability with concanavalin A, and alters their plasma membrane ultrastructure¹¹.

Using syncytium formation as an objective measure of cyclic AMP effect on membrane structure, we have demonstrated clearly that inhibitors of transcription (actinomycin D and cordycepin) or translation (cycloheximide) block the cyclic AMP-mediated membrane phenomena in XC cells (Fig. 1). Table 1 summarises our experience with various cyclic nucleotides and phosphodiesterase inhibitors with respect to their ability to induce fibroblast morphology and to mediate inhibition of syncytium formation, as well as the ability of inhibitors of transcription and translation to block the effect of cyclic AMP on syncytium formation. Only cyclic AMP and its dibutyl derivative were effective. Although dibutyl cyclic AMP is effective alone, cyclic AMP requires theophylline to mediate the morphological and syncytial responses. Non-toxic concentrations of theophylline were ineffective but the more potent phosphodiesterase inhibitors, 1-methyl-3-isobutyl xanthine¹⁵ and analogues of 4-(3,4-dimethoxybenzyl)-2-imidazolidinone¹⁶ mimic the effect of cyclic AMP and dibutyl cyclic AMP, indicative of at least a partially functional adenyl cyclase. Inhibitors of transcription¹⁷ and translation block the membrane phenomena observed with both cyclic AMP and phosphodiesterase inhibitors, suggesting a common process requiring *de novo* mRNA synthesis.

Chemical probes of the mechanism of the cyclic AMP-mediated effect on transcription involved quantitation of histone and acidic (non-histone) protein content as well as activities of the nucleolar and chromatin-associated DNA-directed RNA polymerases. Figure 2 compares the histone and acidic protein with DNA ratios of a normal rat fibroblast in culture, the XC cell, and the XC cell cultivated in the presence of cyclic AMP and theophylline or theophylline alone. The histone levels are comparable in each, although the normal rat fibroblast contains more than twice the content of acidic protein than the transformed XC cell. Growth of XC cells in cyclic AMP did not alter acidic protein levels. Since the chromatin acidic proteins have been implicated in the mediation of steroid responses in certain target tissues¹⁸, as well as in the regulation of gene expression^{19,20}, the acidic proteins of the XC cell as a function of cyclic AMP treatment were examined. SDS gel electrophoresis (Fig. 3) reveals substantial qualitative changes between the normal fibroblast and the transformed XC cell. However, cyclic AMP failed to alter the SDS pattern of the XC cell acidic proteins. Thus at this level of detection, cyclic AMP does not seem to alter the species of chromatin acidic protein present in the XC cell nucleus in spite of the obvious alterations between the normal skin fibroblast and the transformed XC cell.

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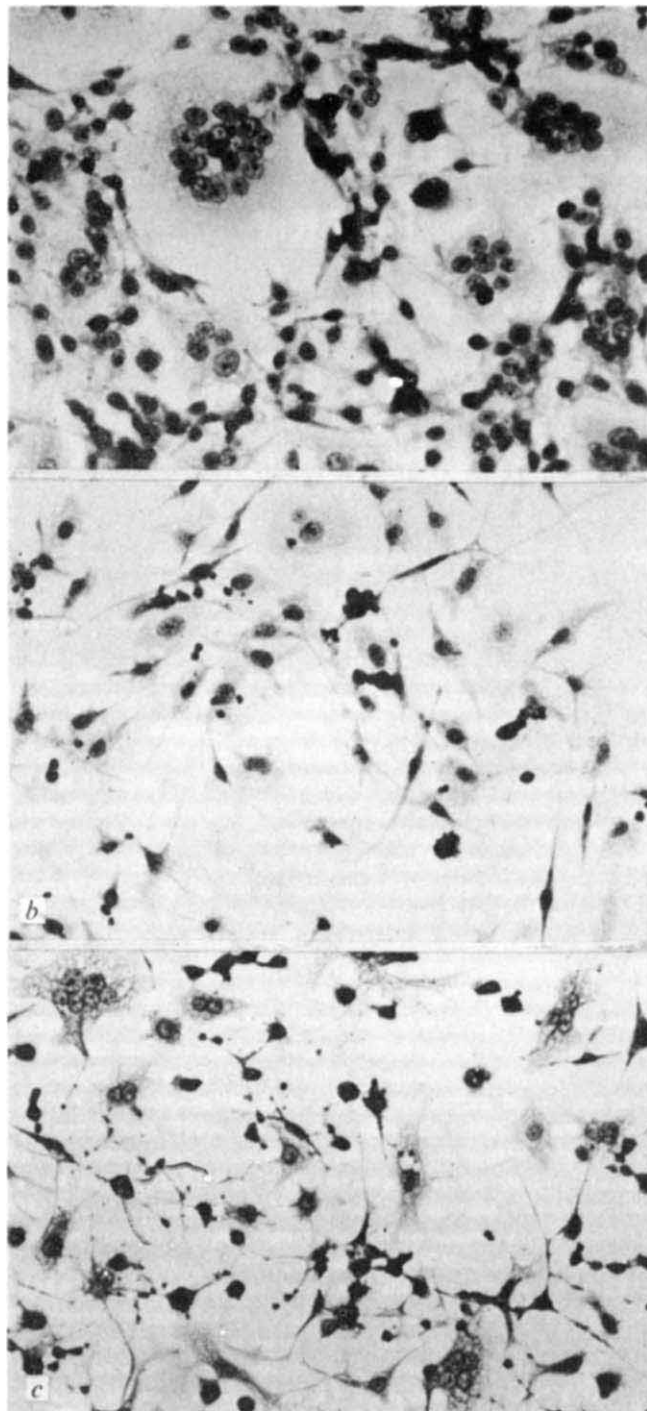


Fig. 1 Syncytial cell formation. Cells were cultivated as previously described²⁷, fixed in methanol and stained with haematoxylin. *a*, Syncytia formed after addition of MuLV producer cells (Y-1 carcinoma) (10^5 cells ml^{-1}) to rat XC sarcoma cells (10^6 cells ml^{-1}). *b*, Assay as in (*a*) except XC cells were incubated in cyclic AMP (3 mM) plus theophylline (1 mM) for 24 h before addition of MuLV producer cells. *c*, Assay as in (*b*) except that cordycepin¹⁷ ($50 \mu\text{g ml}^{-1}$) was added to the XC cell culture medium 24 h before addition of MuLV producer cells.

Endogenous nucleolar RNA polymerase activities were equivalent between normal skin fibroblasts, XC cells and the cyclic AMP-treated XC cells (Fig. 4). However, RNA polymerase II activity (DNA-like RNA transcription) was 50% that of the normal skin fibroblast. Theophylline inclusion in the growth media had no significant effect on activity but the inclusion of 3 mM cyclic AMP resulted in polymerase II activity similar to the normal fibroblast and significantly

greater than the untreated XC cell. Moreover, this increase in endogenous activity accompanied a 50% reduction in the amount of open chromatin template available for *in vitro* transcription (Fig. 4). Thus the inhibition of certain cyclic AMP-induced phenomena in XC cells by inhibitors of tran-

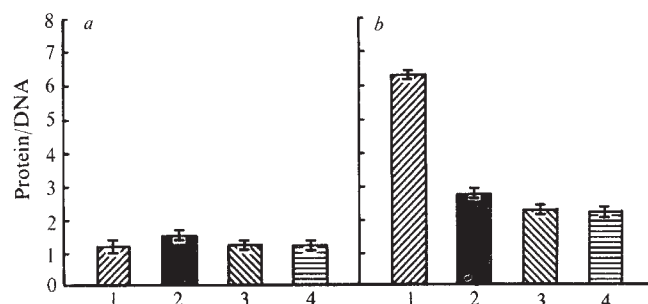


Fig. 2 Comparison of the nuclear histone (*a*) and acidic protein (*b*) composition of normal rat skin fibroblasts (derived from out-growths of Wistar rat skin in organ culture) and XC cells. Growth medium was minimal essential medium with 10% foetal calf serum plus 3 mM cyclic AMP and 1 mM theophylline where indicated. Cells were removed by scraping and then frozen at -20°C . Nuclei were isolated at 4°C by mild cell breakage in a Teflon pestle-glass homogeniser (0.005 mm clearance) in 50 volumes (ml per ml packed cells) of 2.0 M sucrose containing 10 mM Tris, 2 mM MgCl_2 , 25 mM KCl, and 0.1% (v/v) Triton X-100 at pH 7.5 (TKM buffer), and sedimented in a Beckman 60 Ti rotor at $60,000g_{av}$ for 60 min. The nuclei were resuspended in 0.5 M sucrose-containing TKM buffer and filtered through 100 mesh organza cloth and centrifuged for 10 min at $10,000g_{av}$. These nuclear pellets were then extracted with hypo-osmotic buffer to isolate histone and nucleic-acidic protein species as previously described²⁸. 1, Normal fibroblast; 2, XC cell; 3, XC cell + cyclic AMP + theophylline; 4, XC cell + theophylline.

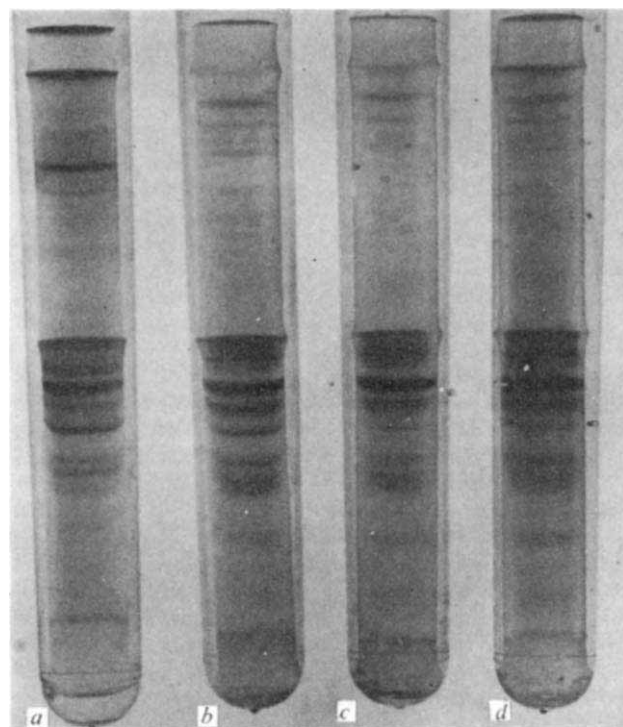


Fig. 3 SDS gel electrophoresis of the chromatin acidic proteins of normal rat skin fibroblasts and XC cells. The total chromatin acidic proteins were isolated and analysed on SDS-polyacrylamide gel electrophoresis by the method of Wilson and Spelsberg²⁹. All nuclear preparations were purified to similar degrees of cleanliness as judged by phase microscopy to avoid cytoplasmic contamination. *a*, Normal rat skin fibroblasts; *b*, XC cells; *c*, XC cells cultivated 3 d in 3 mM cyclic AMP plus 1 mM theophylline; *d*, XC cells cultivated 3 d in 1 mM theophylline. The stain used was Coomassie blue.

Table 1 Effect of Various Nucleotides and Phosphodiesterase Inhibitors on XC Cell Morphology, Ability to Form Syncytia, and their Blockade by Specific Antibiotics

Agent	Morphological reversion	Indicated agent only	Syncytia formation		
			Actinomycin D (5 $\mu\text{g ml}^{-1}$)	Indicated agent plus Cordycepin (50 $\mu\text{g ml}^{-1}$)	Cycloheximide (100 μM)
(A) Nucleotides					
None	—	+	+	+	+
cyclic AMP	+	—	+	+	+
dibutyl cyclic AMP	+	—	ND	ND	ND
cyclic CMP, cyclic GMP	—	+			
cyclic UMP, or cyclic dTMP					
AMP, ADP, or ATP		+			
(B) Phosphodiesterase inhibitors					
Theophylline (1 mM)	—	+			
1 Me-3 iso bu xanthine (0.1 mM)	—	+			
1 Me-3 iso bu xanthine (1 mM)	+	—	+	+	+
Dime-benzyl-2-imidazolidinone (2 mM)	+	—	+	+	+
Bu, me benzyl-2-imidazolidinone (0.1 mM)	+	—	+	+	+

All nucleotides were incubated in the presence of theophylline (1 mM) at a concentration of 3 mM with the exception of dibutyl cyclic AMP which was incubated at 0.1 mM in the absence of theophylline.
ND, not determined.

scription and translation seems to be linked to the activity of the RNA polymerase II synthesising DNA-like RNA.

Postulated Mechanism

Cultivation of XC cells in cyclic AMP or potent phosphodiesterase inhibitors results in the dramatic alteration of various morphological and biochemical processes which seem to have a common origin related to the intracellular concentration of cyclic AMP. Our evidence strongly indicates that the cell surface modifications induced by cyclic AMP and apparently shared by normal cells require *de novo* mRNA synthesis which is associated with an increase in DNA-directed RNA polymerase II activity. Although we have no direct evidence concerning the mechanism of the observed cyclic AMP-induced increase in RNA polymerase II activity, there are several possibilities. These include: (1) induced synthesis of *de novo* RNA polymerase II, (2) removal of inactivation of an

RNA polymerase II inhibitor, (3) direct activation of RNA polymerase II, or (4) indirect activation of RNA polymerase II by a secondary effector such as may occur from new synthesis; phosphorylation of sigma factor by cyclic AMP-dependent protein kinase; or conformation-induced changes in an effector. As far as we know, the only precedents concern indirect mechanisms through a secondary effector. Both phosphorylation of sigma factor of bacterial RNA polymerase by cyclic AMP-dependent protein kinase²¹ and activation of a cyclic AMP binding protein which binds to bacterial RNA polymerase²² are thought to enhance the binding of the polymerase to promoter sites on DNA²²⁻²⁴. Regardless of the mechanism of RNA polymerase activity increase, the facts that cell surface modification induced by cyclic AMP depend on mRNA transcription, are reversible and short lived^{11,25}, and are closely related to the cell cycle of the normal cell²⁶, suggest that the universal property of cell division may be regulated by the relative transcriptional activity of one or more genes continuously producing mRNA, which govern properties of the cell surface.

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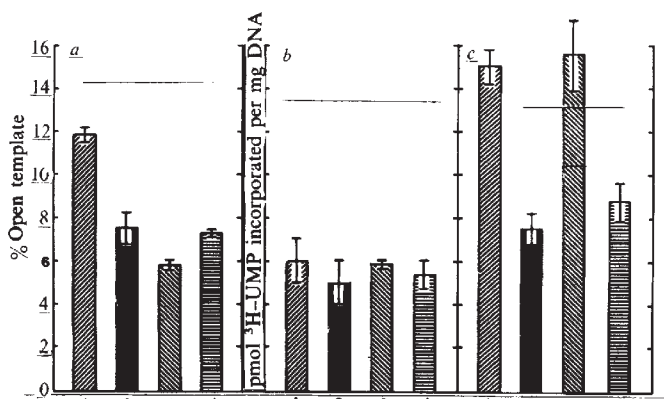


Fig. 4 Comparison of the endogenous DNA-directed RNA polymerase activities from nucleoli and nuclei as a function of chromatin template capacity. Percentage open template was assayed using *E. coli* RNA polymerase and chromatin DNA under conditions where the template is rate limiting as described elsewhere²⁹. Endogenous RNA polymerase activity was assayed as previously described^{30,31} under conditions of low salt (10 mM KCl) containing 0.2 μg α -amanitin or high salt (250 mM $(\text{NH}_4)_2\text{SO}_4$) which differentially distinguishes polymerase I (nucleolar) from polymerase II (chromatin-associated). a, Open template; b, nucleolar RNA polymerase (low salt); c, chromatin-associated polymerase (high salt). Columns are numbered as in Fig. 2.

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Origin of the West Philippine Basin

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Several alternative modes of development for the West Philippine Basin have been suggested during the past few years. The results of Deep Sea Drilling Project leg 31 indicate that none of these speculations is correct and that the basin probably formed by complex pulses of extension during the early Tertiary. A striking lineament which trends north-west across the basin, variously called the Central Basin Fault or Philippine Ridge, had previously been identified as an extinct spreading ridge of Mesozoic to early Tertiary age. On the contrary, this feature is probably a strike slip fault, possibly with a left-lateral displacement.

Among these, the West Philippine Basin is the largest, and, at an average depth of nearly 6 km, approaches the depth of the mid-Mesozoic western Pacific basin. Because this great depth should imply great age¹, several recent papers²⁻⁶ have speculated that the West Philippine Basin is Mesozoic or even older, or possibly represents a section of the Pacific basin isolated by the development of ensimatic island arc systems.

We report here the results of Deep Sea Drilling Project leg 31 in the Philippine and Japan Seas (Fig. 1), as they pertain to the origin of the West Philippine Basin. These data, coupled with other unpublished information (profiles and bottom sample from Antipode cruise of Scripps Institution of Oceanography), indicate that the West Philippine Basin has not been generated as part of the Pacific basin as suggested, but is a complex early Tertiary basin developed at least in part behind a pre-existing arc system. Unfortunately, however, the possible alternative origins have only been reduced by the rejection of several possibilities. The answer remains to be gleaned from future detailed, problem-oriented studies in the region.

One speculated mode of origin which has attracted a number of recent adherents is that the West Philippine Basin was generated by crustal spreading along the Central Basin Fault (Fig. 1), either within the Pacific Basin or behind an arc system. The Central Basin Fault (profiles and bottom sample from Antipode cruise of Scripps Institution of Oceanography and refs 7 and 8) is a north-westerly trending lineament which

THE origin of the many and varied marginal basins along the rim of the western Pacific continues to provoke lively discussion.

cuts diagonally across the basin from the Palau-Kyushu Ridge to the Palau Ridge between Luzon and Taiwan⁹. Although not explored in detail, the fault is actually a zone of very rough topography with troughs over 6.5 km deep and ridges shallower than 3.5 km. A brief local survey and available regional bathymetry suggests that the individual ridges are discontinuous and may not parallel the zone itself. At its south-eastern end, the Central Basin Fault appears to splay to a zone 300 km wide which may be curving southwards and intersecting the Palau-Kyushu Ridge at a low angle (D. E. Karig, unpublished bathymetric chart).

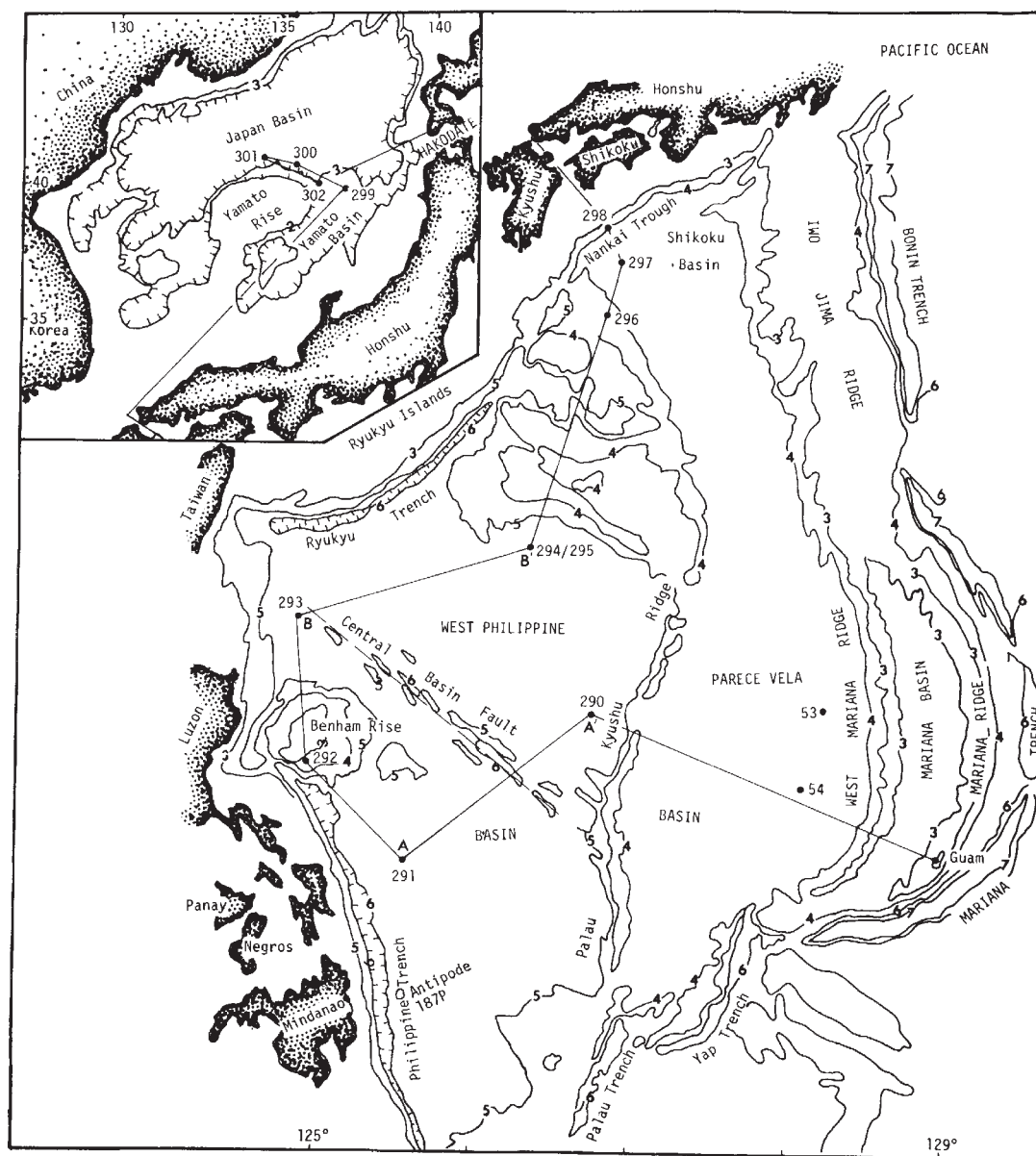
A bathymetric profile published by Ben-Avraham *et al.*³ is apparently only fortuitously similar to those across active spreading centres and is not typical of most parts of the Central Basin Fault (Fig. 2). Even in their profile, there is a discontinuity at the edge of the zone of very rough topography rather than the smooth concave upward curve characteristic of a spreading centre. Similarly, the gravity anomalies can be related simply to isostatic compensation of the larger topographic features. Their magnetic profile, which has apparent symmetry, is again not typical of other crossings of the Central Basin Fault (Fig. 2). Furthermore, brief surveys at Deep Sea Drilling Project sites 290, 291, 292, 293 and along the AT and T cable route between Guam and Manila¹⁰ show that magnetic

and topographic lineations do not parallel the Central Basin Fault, but trend NNE or less commonly NNW.

If the West Philippine Sea were Mesozoic in age and resulted from late Mesozoic spreading on the Central Basin Fault^{4,5} then the demonstrably widespread early Tertiary basalt cover must have had a very low and uniform remanent magnetism so as not to mask or disorganise the older magnetic field. The unlikelihood of such magnetic conditions, the incompatible magnetic and topographic trends, and the lack of evidence for any Mesozoic basin crust lead us to dismiss such an origin.

If, on the other hand, the crust of the basin spread from the Fault in the early Tertiary¹¹, the most plausible fit to the drilling results calls for a half rate of 2.25 ± 0.25 cm yr⁻¹ with the termination of spreading near 30 m.y. ago (Fig. 3). In this scheme, the basement of the Benham Rise would have developed after the basin formed, and reworked Eocene nannofossils found at the base of hole 293 and close to the fault would have been derived from some 300 km to the south-west from an older part of the Philippine plate which has not been subducted. At least the latter assumption is unlikely. Even so, the resulting magnetic pattern cannot be fitted to the mid-Tertiary magnetic stratigraphy and the thickness of pelagic sediment adjacent to the Central Basin Fault is too thick for a Late Oligocene age².

Fig. 1 Sketch bathymetric chart of the Philippine Sea, with track and drill sites of DSDP leg 31. Sites 53 and 54 are from DSDP leg 6; Antipode 187-P is an SIO piston core. The magnetic and bathymetric profiles on Fig. 2 are along the track between sites 290-291 and 293-294. Depths are in km.



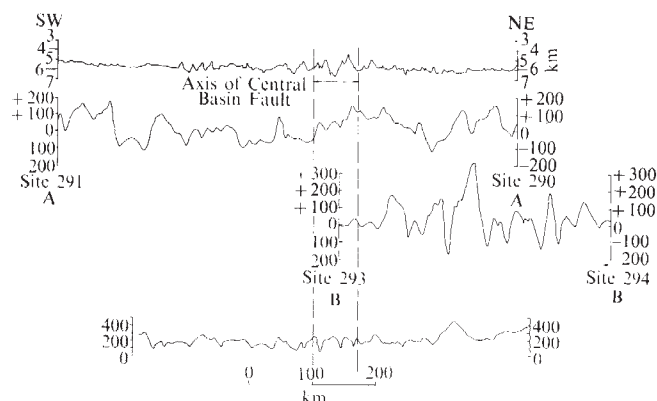


Fig. 2 Magnetic and bathymetric profiles across the Central Basin Fault in the West Philippine Basin. Profiles A-A' and B-B' are from DSDP leg 31. The lower profile is from ref. 3. All profiles have been projected normal to the Central Basin Fault, which trends 305° , and stacked so that the fault axis is represented by the vertical band. No correlation or analyses are, however, attempted.

A fit of basement ages through holes 290, 291 and 293, again assuming a younger age for the Benham Rise, would demand a pulse of spreading in early Late Eocene at a half rate of 29 cm yr^{-1} . Such spreading rates far exceed the most rapid so far postulated¹², and the corresponding Eocene magnetic pattern would have a much longer wavelength than those observed.

The lack of supporting data and the contradictory evidence presented here effectively preclude the identification of the Central Basin Fault as a spreading ridge of Tertiary or Mesozoic age. It seems more likely that, as Hess⁷ originally postulated, the fault is strike-slip in nature and probably originated as some sort of lithospheric transform. The straightness of the fault trace, the probable en echelon internal geometry, and the lack of a regional vertical displacement across the fault are the strongest lines of evidence supporting strike-slip activity.

The sense and amount of displacement are not clear, but the apparent truncation and offset of segments of the Palau-Kyushu Ridge suggest left-lateral slip of up to several hundred kilometres. Because the fault cuts the Late Eocene volcanoclastic apron drilled at site 290 and because the basement below is probably mid-Eocene, the maximum age of displacement is bounded. At the north-west end, the truncation of fault structures by a Palaeogene subduction zone⁹ and overlap by a Middle Miocene or younger sedimentary apron provide an upper limit of fault activity. The tectonic role of the fault is unknown, but it may somehow be related to the jamming of the early Tertiary west-dipping subduction zone along the eastern Luzon coast by the Benham Rise. A similar relationship has been postulated between the Ontong Java Plateau and a trench north of the Solomon Islands¹³.

Another postulated origin for the West Philippine Basin is that it represents part of the western Pacific basin trapped behind a younger arc system and modified by later volcanic activity³. This idea seems to have been engendered by the similarities in water depth and heat flow with those of the Pacific Basin and also by a faint indication of increasing thickness of the second crustal layer eastwards across the Philippine Sea. Several other observations made during Deep Sea Drilling Project leg 31 might be marshalled in support of a trapped, modified crust. The occurrence of non-calcareous brown clay lying on basalt at site 291 and possibly at site 294 suggests that the basalt was extruded below the calcium carbonate compensation depth and well after the basin crust was generated at a shallower depth. The close grouping of basement ages near Mid and Late Eocene in holes 290, 291, 292 and possibly 293 could also be interpreted as a result of widespread, flood-like eruptions.

One would, however, expect that widespread basalt flows

would either leave a few windows through which the older section could be seen, or else that the volcanic cover should be thick enough to be discerned on seismic refraction profiles. There is no place on the rather extensive reflection profile data in the West Philippine Basin where any older, uncovered section can be seen. Neither is there any evidence of a masked layer situation on seismic refraction profiles^{3,14}. To the contrary, the available data show a second layer which is as thin as or thinner than that of normal oceanic crust. There is literally no room for the insertion of a masked layer and a double basement in the crust of the West Philippine Basin.

Although the apparent re-occurrence of a Middle to Late Eocene basement age in the holes drilled in the West Philippine Basin was surprising, the total observed age spread was at least from Palaeocene (60 m.y.) to Late Eocene (40 m.y.). Volcanic activity persisting this length of time is more easily associated with crustal generation than with a pulse of subsequent volcanism.

The lack of calcareous sediments immediately above the basalt remains a puzzle. The basal calcareous brown clay of Palaeocene age at site 295 may reflect the generation of crust in the Philippine Sea when the calcium carbonate compensation depth was nearly as shallow as the crest of the spreading ridge¹¹ and beneath waters of low productivity. The relatively rapid deposition of volcanically derived brown clay at depths of 3 to 3.5 km in the Mariana Basin is presently producing such a section¹⁵, but this clay is ash-rich whereas the sections cored at sites 291 and 294/295 are not.

A variation on the trapped oceanic crust theme would have the crust of the West Philippine Sea generated along the spreading system which produced the late Mesozoic anomalies east of Japan and which has since been subducted^{12,16}. The section of the spreading ridge which lay in the area to become the West Philippine Basin has presumably been subducted or remains unidentified. This early Tertiary crust would have then been trapped behind an arc system whose age would be determined by the basal late Middle Eocene sediments on the Bonin Islands¹⁶, Siapan¹⁷, Guam¹⁸ and Palau¹⁹.

The major problem with this interpretation is that the crust in the West Philippine Basin at sites 290, 291, 292 and 293 is no older than the arcs and may even be younger. The rather mature frontal arc which reached sea level in many areas thus had very little or no time in which to develop. A suspected pre-Tertiary history of the arc systems might be read from the few Late Cretaceous planktonic foraminifera collected from the volcanoclastic apron west of the Palau-Kyushu Ridge (site 290) and supposedly derived from that ridge. Finally, there may have been as much as 45° of clockwise rotation of the Philippine

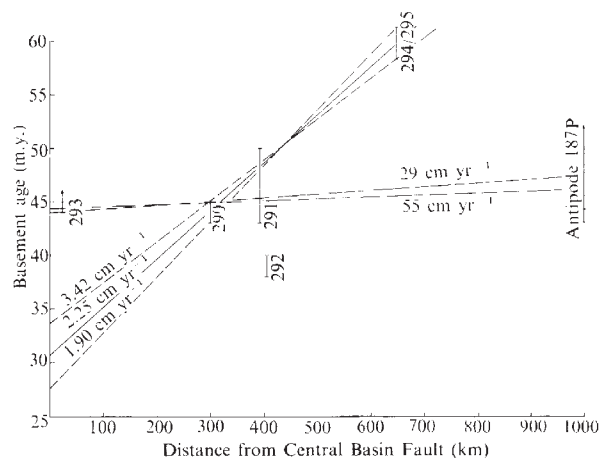


Fig. 3 Plot of basement age versus distance from the Central Basin Fault using data from DSDP leg 31 and Antipode 187-P. Shown are the range of possible half-spreading rates which could be fitted to the data. The solid bars indicate the likely range in basement age; arrows indicate minimum ages.

Plate relative to the Asian and Pacific plates during the Tertiary²⁰, but this is still a rotation insufficient to convert the east-northeast magnetic lineations east of the Japan and Bonin trenches to north-south lineations seen in the Philippine Sea.

The West Philippine Basin might instead have been produced by some form of crustal extension behind a pre-existing arc system. Within the scope of this hypothesis, extension could have occurred symmetrically, asymmetrically or in a more disorganised fashion within active marginal basins. The smaller, presently active marginal basins which have been investigated are spreading symmetrically^{9,21-23}, but if this mode continued in a basin as large as the West Philippine Basin, then a symmetrical spreading centre would no longer be located above the Benioff zone with which it is assumed to be associated.

In the West Philippine Basin the dominant north-south lineations imply an east-west spreading direction but it is difficult or impossible to choose a north-south spreading ridge which would be compatible with the known basement ages. The widespread distribution of Late Eocene basement also would require improbable rates of extension. It is therefore unlikely that a single, simple pulse of inter-arc basin extension formed the West Philippine Basin.

A new look at the West Philippine Basin suggests a more complex history, involving separate generations of subunits, some of Palaeocene or even greater age. The abundance of late Middle to Late Eocene basement in the basin and basal sediments on the surrounding arcs is indicative of a major tectonic event at that time. The northern sector of the basin seems to be older and might be related more closely to the development of the Daito-Oki Daito ridge complex which unfortunately has, so far, received very little attention.

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Molecular Model for Elastin Structure and Function

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Amino acid sequences in elastin contain striking patterns. These are interpreted in terms of possible molecular conformations, and an 'oiled coil' model is proposed to explain the protein's elastic behaviour.

The structure-function relationships in several fibrous proteins such as collagen^{1,2}, keratin³, and silk fibroin^{4,5} are fairly clear. Elastin is an exception in spite of its role in the elasticity of

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arteries, lungs, and skin, chiefly because of its lack of a good X-ray diffraction pattern^{6,7} and its extreme insolubility hindering sequence analysis. It is usually regarded as a gel of randomly coiled peptide chains, cross linked to each other^{8,9}, and attempts have been made to apply classical rubber theory to its behaviour¹⁰. Weis-Fogh and Anderson have reported^{11,12}, however, that the thermodynamics of stretching do not fit those of a normal rubber, there being strong interactions between the protein and the swelling agent (water). They therefore proposed a 'liquid drop' model, in which spherical monomers are cross linked to form a three-dimensional aggregate (compare with ref. 13). In this model, the few hydrophilic amino acids are scattered over a hydrophobic surface, and any deformation of the subunits leads to a larger hydrocarbon-water interface. This model explains very well the thermodynamics of the stretching, in which for

a small negative free energy change (imposed stretching) there is a large evolution of heat and a high negative entropy change^{11,12}.

We have analysed amino acid sequences in a soluble elastin, isolated from aortae of copper-deficient pigs¹⁴. Several striking features of these sequences lead us to propose a new model for elastin, which, like the liquid drop model, emphasises hydrocarbon-water interactions, but views the monomeric units as fibrillar rather than globular. In our model, the principal site of hydration is the peptide chain rather than amino acid side chains. In this way, we can readily explain the high water content of elastin (50–60% by weight^{8,15}), and reversible transition to a brittle, inextensible material on drying⁷.

In spite of striking similarities in amino acid compositions, elastin and collagen seem unrelated in sequence. Hydroxyproline occurs in elastin, its principal locations being those where the local sequence resembles those that are hydroxylated in collagen (-Gly.X.Pro.Gly-).

Soluble Elastin

Mature elastin is too insoluble for extensive sequence analysis because of cross links formed from oxidised lysine residues^{16,17}. Copper-deficient animals fail to make cross links, and a soluble elastin can be isolated¹⁴. Soluble elastin from pig aorta has a chain length of 800–850 residues, based on a molecular weight of approximately 70,000 (refs 14 and 18); this is compatible with our studies of tryptic peptides^{19,20}.

Table 1 Amino Acid Composition of Soluble and Mature Elastin

		Residues per 850 residues*	
		Soluble	Mature
Gly	G	275	277
Ala	A	196	197
Pro	P	95	80
HyPro	P	7.5	12
Val	V	110	105
Ile	I	15	16
Leu	L	38	44
Tyr	Y	12	14
Phe	F	24	27
Arg	R	4.4	5.5
Lys	K	38	6
Cross links †		Very low	25–30
Asp + Asn	D + N	2.7	7.5
Thr	T	12	14
Ser	S	8	11
Glu + Gln	E + Q	16	18
Met + Cys + Trp + His		0	2.6

* Based on an assumed chain length of 850 residues.

† Expressed as Lys equivalents.

Modified from refs 14 and 33.

Loss of lysine and formation of cross links are the chief differences seen by amino acid analysis of soluble and mature elastins (Table 1). Notable features of the composition *per se* are: the high content of Gly, Ala, Val and Pro; the scarcity of Arg relative to Lys; absence of His, Cys, Met and Trp; and the very low frequency of polar amino acids (less than 5% total).

Amino Acid Sequences in Soluble Elastin

Sequential degradation^{20,21} of intact protein reveals one major N-terminal sequence (Table 2), plus a rather high background (10–20%).

We have previously reported the occurrence of multiple copies of the sequences -Lys.Ala.Ala.Lys- and -Lys.Ala.Ala-

Ala.Lys- in soluble elastin²² and an enrichment of Ala close to the C-terminal Lys of large (17–110 residues) tryptic peptides²³. N-terminal sequences of the latter^{20,21} are shown in Table 2.

Patterns Observed in the Sequences

Our data reveal two basic types of region in elastin: Ala- and Lys-rich areas, presumably concerned with cross linking; and Gly-, Pro-, and Val-rich areas, presumably concerned with extensibility. We do not yet know if there are many short stretches of these or a few larger ones. Within each type the observed sequence patterns suggest models for structure-function correlations.

In the cross link regions, the pairwise association of lysines and extreme enrichment in Ala are very striking. Unsequenced C-terminal portions of large tryptic peptides are enriched in Ala relative to the whole peptides; peptide T7b contains eight consecutive alanines preceding its C-terminal Lys (Table 2).

In the extensible regions, regularities occur at three levels: (1) nearest-neighbours; (2) short-range repetitions; (3) long-range repetitions. These may not be independent, as manifold repetition of a short sequence gives rise to otherwise improbable nearest-neighbour relationships, for example.

Nearest-neighbour analysis of data from Table 2 is summarised in Table 3, excluding small peptides. The principal features are: Pro is usually followed by Gly (31/36), but rarely preceded by it (4/39); large aliphatic residues are normally preceded by Gly (62/74).

Short-range repetitions of sequence are a most notable feature of the data (underlined in Table 2). The limited divergence within peptides T1, T2 and T7a suggests that some of the local duplications are recent. In other peptides there are suggestions of regularity, more or less obscured by amino acid substitutions and deletions. The elastin gene could be considered as a microcosm of the whole DNA, containing families of repeated sequences varying in ages and degrees of relation²⁴. Multiplication of short sequences could be a powerful force in the evolution of structural proteins because of the speed with which radically new properties can appear. Silk fibroin sequences such as (-Ser.Gly.Ala.Gly.Ala.Gly-)_n are closely related to elastin sequences such as (-Pro.Gly.Val.Gly.Val.Ala-)_n. A few point mutations, plus genetic amplification, can lead from one structure to the other.

Long-range repetitions are suggested by marked similarities between different peptides, but cannot be defined accurately until more is known of the order of peptides in the molecules. A plausible mode of evolution of elastin involves duplication of large segments each containing a cross link site and some extensible region. Present evidence suggests there may have been a six- or eight-fold multiplication.

Molecular Models for Elastin

The regularities of primary structure prompted us to examine possible regular structures for elastin. For this we used CPK atomic models²⁵ and conformational maps²⁶ to help avoid too-close contacts. The cross link regions will be discussed only briefly as a full discussion is in preparation (W. R. G., L. B. S. and J. A. F., manuscript in preparation).

In the cross-link regions, pairs of lysines occur in sequences such as -Ala.Ala.Ala.Lys.Ala.Ala.Lys.Tyr.Gly.Ala.Ala-; the sequences could be much longer, with more lysines and with longer runs of alanine at the amino end. Such regions would favour an α -helical conformation, with lysines protruding from the same side of the helix; this facilitates intrachain condensations between oxidised lysines. Subsequent interchain condensations lead to desmosine and isodesmosine linking two α -helical chains, with very little strain. Estimates of helical content of hydrated elastin vary from 10–25%, depending on the model system used and on correction factors^{27,28}. Cross-link areas might be expected to contribute 15–20% α -helix.

Table 2 Amino Acid Sequences in Soluble Elastin

Large tryptic peptides*										
	1	5	10	15	20	25	30	35	40	45
T 15 (88 residues)	G G V P G A V P									
Whole protein	<u>G G V P</u> G A V P <u>G G V P</u> <u>G G V F</u> F P G A G L G G L G									
T 1 (85 residues)	(K) Y G A A G G L V P G A P G F G P <u>G V G V</u> P <u>G V G V</u> P <u>G V G V</u> P <u>G V s</u> V <u>p</u> <u>G V g</u> V <u>P</u> <u>G V g</u> v									
T 4 (110 residues)	(K) Y G A P G A G V L P G V G V G G V g v p g									
T 9c (30 residues)	(K) Y G A A G A L G G V G B L G G A G I P g x V A G									
T 2 (60 residues)	A A Q F G L G P <u>G I G V A</u> P <u>G V G V A</u> P <u>G V g</u> V A <u>P G V G V a</u> P <u>G V G V A</u> P x I									
T 9b (36 residues)	A A E F G V G G V G G L x V G G L G A V x G A G									
T 6 (40 residues)	A G A G L G G V G G V G G L G v s T G A V V P									
T 14b (39 residues)	A P G G G G A F A G I P G V G <u>P</u> F G G Z Z P G V P L x G p									
T 7a (58 residues)	(R) G G V G V G G I P T <u>F G V G A G G F</u> <u>P</u> <u>G F G V G V G G V p</u> G A A L									
T 12 (24 residues)	V P G V G L <u>P</u> G V Y P G									
T 14c (17 residues)	(R) F P G V G V L P G V P T G T G V K									
T 7b (21 residues)	A G Y P T G T G V G T Z A A A a A A A K									
Small tryptic peptides† (Mol per mol protein)										
A A A K (6)	A P G K (2)									
A A K (6)	A K (1)									
S A K (2)	Y G A R (2)									
C-terminal fragments of large tryptic peptides‡										
A K (7.3)										G K (0.5)
A A K (4.5)										G V K (1.2)
A A A K (0.9)										G P K (0.5)
G A K (0.8)										G A R (1.3)

Abbreviations as in Table 1; B, Asp or Asn; Z, Glu or Gln; x, unknown; lower case letters indicate residues that are identified with a lower degree of confidence. Only the principal hydroxylation sites are indicated as P.

* Ref. 20, plus some previously unpublished data. All except T14c and T7b are partial sequences.

† Ref. 22.

‡ Ref. 23. Results are expressed as mole per mol protein, based on assumption that all fragments were recovered in equivalent yield. (Measured overall yield was 70%.) The method used underestimates presence of sequences such as A A K, since some appears as A A K and A K.

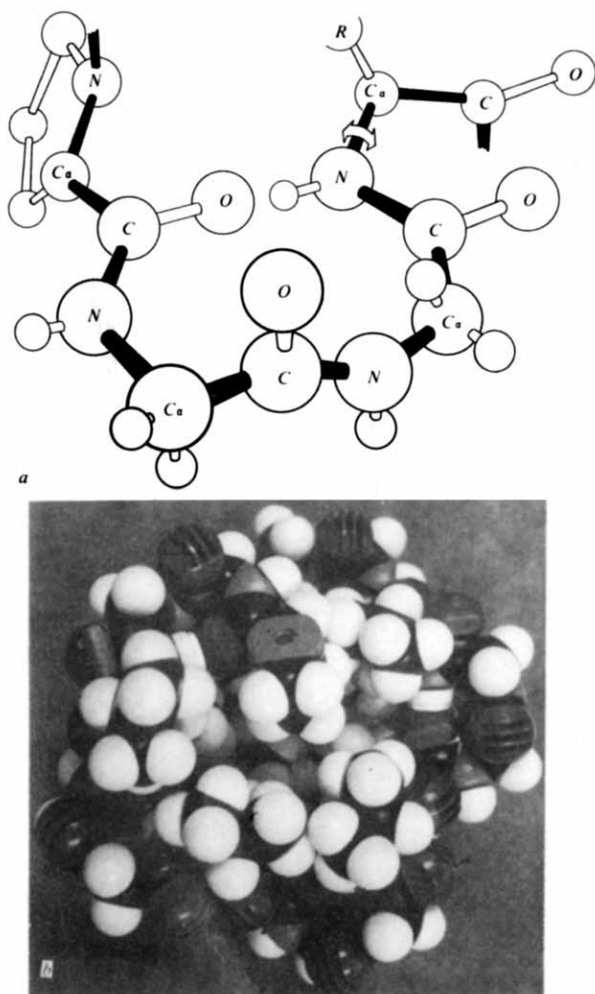


Fig. 1

Regions between cross links are depleted in Ala and rich in Gly, Pro, and Val; these presumably control the protein's extensibility. Two extreme views can be held about the chains here. In one, the chain is randomly coiled and kinetically free, as in 'classical' rubbers^{8-13,15}; in the other, the chain has a preferred conformation that is returned to after release of a stretching force. Probably a dynamic blending of the two is most realistic.

Measurements of heat changes during reversible stretching

Table 3 Nearest-neighbour Relationships in Elastin

First residue	Second residue				
	Gly	Ala	Pro	Val + Leu + Ile	Others
Gly	22	16	4	62	9
Ala	12	12	8	5	4
Pro	31	0	0	1	4
Val + Leu + Ile	36	6	21	4	5
Others	14	2	6	2	7

Compiled from data of Table 2, excluding the small peptides and fragments.

suggest strongly that hydrophobic groups are forced into water during stretching and that energy for contraction comes from the return of these groups to a non-polar environment^{11,12}. We propose that these interactions are controlled by definite conformations of the protein, and that the observed sequence patterns reflect the constraints imposed by the secondary structure.

We have discovered some plausible structures for the amino-terminal type of repeat that immediately suggest a working model for the extensibility of elastins. Other repeat sequences can assume conformations having the same general properties. The main feature of our model is a broad coil in which glycines occupy the exterior positions exposed to solvent, while Pro, Val, and other hydrophobic residues are buried; most of the peptide backbone is hydrated. For descriptive purposes we call this general type of structure an 'oiled coil'.

A β -turn^{29,30} made from the tetrapeptide -Pro.Gly.Gly.Val- is shown in Fig. 1a. It forms a compact block with the bulky hydrophobic groups at one end and the glycines at the other. When several such blocks are linked, they make a broad left-handed helix (Fig. 1b) with bulky side chains in the centre and glycines on the outside; this creates a natural inside and outside in aqueous conditions, the exposed peptide chain being hydrated. No H-bonding occurs along the direction of the helix axis, so the coil is readily extended by rotation around the C α -CO bond of Pro (decrease Ψ) and around the Val C α (decrease ϕ , increase Ψ). A 30° decrease in Ψ for Pro, with appropriate changes for Val, produces an extension of about 2.5 Å for every tetrapeptide unit in the coil, more than doubling the length of such regions. Although extension does not disrupt intrachain H-bonds, it is energetically unfavourable on two counts. First, it exposes the hydrocarbon core of the coil to water, leading to structuring of the water and an unfavourable entropy decrease; this fits the observed thermodynamics of stretching^{11,12}. Second, the conformation of Val becomes less favourable, judged by conformational maps²⁶. Release of the stretching force should thus lead to contraction to the original state.

Beta-turns themselves are not very restrictive, and suitable conformations can be found for almost any group of amino acids³⁰. To produce an oiled coil structure from them, however, puts limitations on the polarity of the various residues. For the structure described, maximum stability requires hydrophobic groups in positions 1 and 4 and polar groups in positions 2 and 3.

Urry³¹ discussed β -turns as binding sites for calcium in elastin, using peptide antibiotics as models. We feel that conformations with bound calcium are likely to be inside-out with respect to hydrophobicity; this is acceptable for molecules functioning in a non-polar environment such as a cell membrane but not for a hydrated elastin fibre. Binding of calcium would stabilise a rigid inside-out conformation.

Repeating sequences such as (-Pro.Gly.Val.Gly.Val.Alala-)_n and (-Pro.Gly.Val.Gly.Val-)_n were found after we had developed the oiled coil model for the tetrapeptide. So far we have not found β -turn structures for these that have such a clear inside-outside character, but structures other than the β -turn can give rise to the same properties (Fig. 1). In such structures the role of Gly may be as much steric as hydrophilic since it alone allows the chain to fold back so tightly.

Overall View of Elastin Model

Our model for elastin is outlined in Fig. 2. Each monomer is fibrillar, and made up of alternating segments of cross link regions and 'oiled coils'. The mechanical properties of these are quite distinct, being rigid and flexible, respectively. Each monomer is linked to many others, forming a network like a three-dimensional mattress spring. Chains may be cross linked at various angles, or bent, so the networks could be isotropic, despite the fibrillar nature of the monomers.

Our scheme for the process of cross linking makes predictions about the mechanical properties of elastin. Thus 'cross links' formed from two lysine residues are local intrachain knots and not true cross links, and each residue of desmosine and isodesmosine cross links only two chains, rather than the three or four which it is potentially able to. From the known cross link content of mature elastin, therefore, the average chain mass between cross links (M_c) would be about 7,000 daltons; other cross linking schemes give M_c as low as 2,000–4,000 daltons.

The diameter of the 'oiled coils' is about 12–15 Å and the hydrophobic core can accommodate non-polar molecules, especially straight-chain aliphatic compounds. Partridge^{8,9,13} investigated the gel-filtration behaviour of a number of compounds on columns packed with elastin fibres. He found that elastin could be regarded as being made up of randomly disposed hydrated rods about 16 Å in diameter, with free water spaces between them. Aliphatic alcohols showed anomalous behaviour, partitioning preferentially within the gel particles, the effect being greater for *n*-alcohols than for branched. We suggest that they might be fitting within the core of the coils, rather than adsorbing on to the surfaces.

From the composition, we estimate that 20–25% of the molecule should be in cross link regions, with the rest as coil—the two forms then contribute equally to the length of the relaxed monomer. Extension of the coils proceeds smoothly until the whole molecule is 2–2.5 times the original length. Stretching increasingly exposes the hydrocarbon core to water; this should lead to restructuring of the water, accompanied by the thermodynamic changes observed by Weis-Fogh and Anderson^{11,12}.

M_c varies inversely with the elastic modulus of the rubber if it can be assumed that the contractile force is generated solely by entropic diffusion of the macromolecular chains. The stress-strain behaviour of elastin has been measured in various solvents, enabling M_c to be estimated¹⁵. (We use M_c to indicate values of M_c measured from stress-strain behaviour on the assumption of random kinetic diffusion of chains. M_c denotes actual mass of chain between cross links.) In aqueous formamide (40% v/v), M_c reaches its maximum of about 7,200 daltons, close to our predicted value. In these conditions, we expect the protein chains to be completely unfolded so that elastin should behave as an 'ideal rubber' with M_c representing the true mass between cross links. In water, M_c is 3,500 daltons, corresponding to an increased elastic

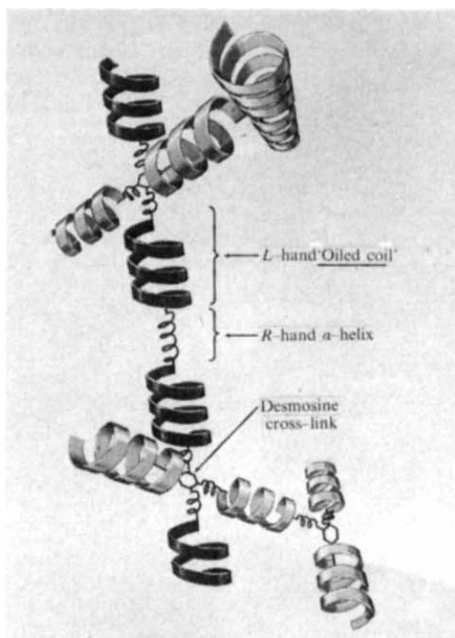


Fig. 2

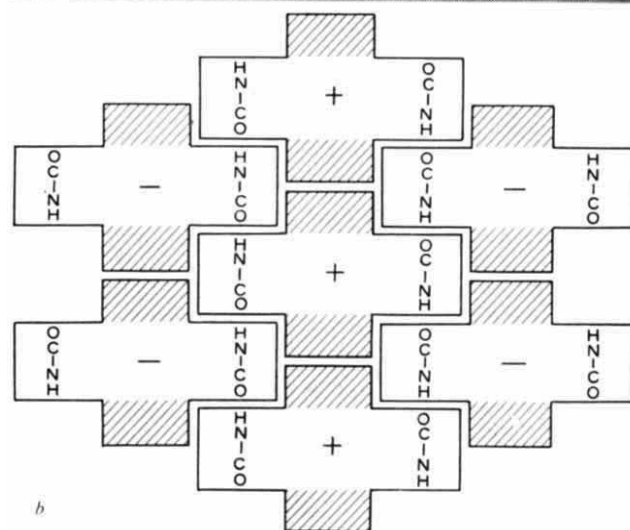
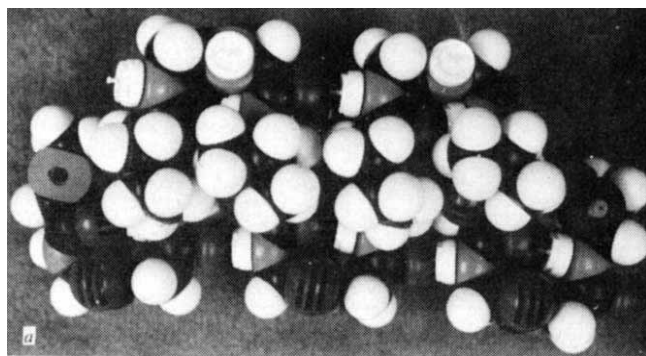


Fig. 3

modulus. We interpret this as being due to two main causes. First, lengths of chain in the cross link areas are rigidly attached to the cross links and not free to diffuse; second, the oiled coils generate an additional contractile force due to the interaction of their hydrophobic groups with water. In conditions likely to disrupt hydrophobic interactions but leave α -helix unaffected (for example, 8% v/v aqueous butanol), M_c is about 5,400 daltons, which corresponds with our estimate of the average mass of the coil regions. These conditions also cause profound changes in the thermodynamic parameters of elastin stretching^{11,12}.

When dried, elastin becomes brittle, its elastic modulus being three orders of magnitude greater⁷ than that of the hydrated form (50–60% water by weight). Such a transition, which is reversible, is remarkably easy to accomplish with our model for the oiled coil form of $-(\text{Pro.Gly.Gly.Val})_n-$. When the Val N-C α bond is rotated by about 180°, the tetrapeptide units stack quite differently, forming a modified cross- β structure (Fig. 3). The repeat distance is four residues, with two H-bonds between neighbouring antiparallel segments of chain (proline residues prevent formation of a third). Peptide bonds in the β -turns are perpendicular to the chain axis so systematic H-bonds form between chains of opposite polarity. Hydrophobic side chains form two ridges that interact between chains of the same polarity (see Fig. 3). Similar cross- β structures can be made from the hexapeptide and pentapeptide, the latter having a repeat period of ten residues. Such a structure, cross-linked by H-bonds, would have a much higher elastic modulus than the oiled coil; transition from high to low modulus occurs close to the point (30% water by weight) where there is one water molecule for each peptide carbonyl and amide group.

Our general model for elastin thus provides a molecular basis for the behaviour of the protein, and clarifies several features that are not readily explained by a model that invokes

only the usual entropic diffusion of randomly coiled chains. It does, however, suggest a static molecule in which chains are coiled in a particular way, and return to the same conformation each time after stretching. Recent ^{13}C -NMR studies, on the other hand, indicate high mobility of the peptide chain in native elastin³². We suggest that this is caused by oscillation and flexion of the network of coils, rather than by completely random segmental motion of its microscopic parts.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Radio Detection of Interstellar CH

As recently as 1968 the only molecules known to exist in interstellar space were CH, CH⁺ and CN, identified by means of their absorption spectra superimposed on the optical spectra of bright stars, and OH which in its ground state emits at a radio wavelength of 18 cm. Since then there has been a remarkable series of discoveries of about twenty additional molecules emitting in the radio spectrum, some of them quite complex (CH₃OH for example). But of the originally discovered 'optical' radicals, only CN, emitting at about 2.6 mm, has been detected at radio frequencies—in the Orion Nebula, in the HII region W51, and in the infrared object IRC+10216. The other radical, CH, has not been found, mainly because the radio frequencies of its ground state lambda doublet have not been found in the laboratory, and it has not been possible to compute them theoretically with sufficient accuracy. The first estimate of the (main line) frequency seems to have been made by Shklovsky¹: $3,180 \pm 10$ MHz.

Two previous searches for CH with the 84-foot radio telescope of the Onsala Space Observatory (Rydbeck and Elldér), in September 1968, covering the range 3,000–3,135 MHz, and in October–November 1971, covering 3,361–3,385 MHz, proved negative².

We resumed the search in October 1973 with an improved rufite travelling wave maser and linear polarisation (zenith system noise 35 K), and have now succeeded in detecting the ground state lambda doublet ($^2\Pi_{1/2}$, $J=1/2$) at a wavelength of approximately 9 cm in the direction of the following objects: W3OH, W3 Cont, W12, Orion A, W31, W43N, W44, W49,

W51, DR21, W75 B, Heiles clouds 2 and 1B, Cudaback–Heiles cloud G84.7–1.0, Lynds cloud L134, and Cas A (Orion and Perseus arms). Since the transition was seen in HI and HII regions, as well as dust clouds, there is reason to believe that CH is widespread in our Galaxy.

CH was seen in emission in all objects, and not in absorption even in Cas A, W12, and cloud G 84.7–1.0, as one might have expected from OH observations. This is especially remarkable in the direction of Cas A, where CH seems to behave like a weak maser. One can only guess about the possible mechanism of inversion of CH in the 'clouds' in the direction of Cas A. Reactions leading to the formation of CH might leave it in an inverted ground state. Collisions with hydrogen atoms could perhaps have the same effect. In the cool dust clouds, on the other hand, where molecular hydrogen is prevalent, such a collisional effect seems less likely.

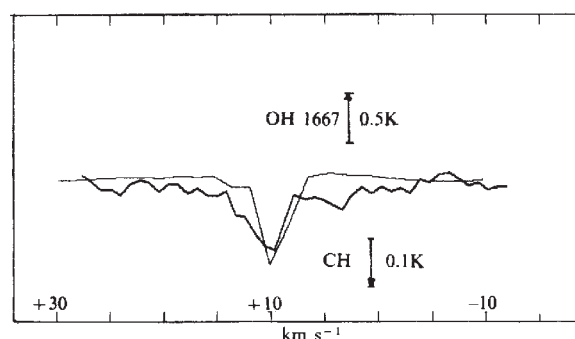


Fig. 1 W12, CH (—) and OH 1667 (RC+LC)/2 (---) spectra.

We have not seen CH in the anomalous, strong emission which is frequently observed for OH in HII regions. Nor was there any evidence of magnetic splitting. In this respect CH seems to resemble OH in its excited $^2\Pi_{1/2}$, $J=1/2$ state.

The CH features are very well correlated with OH main line absorption and thermal emission features. The correlation with the formaldehyde spectra is also quite good. The rest frequency of CH, as determined from comparison with 1,667 OH features in W12, Heiles cloud 2, Lynds cloud L134, and Cas A, is $3,335.475 \pm 0.010$ MHz. The error corresponds to the width of the channels, 10 kHz, of the multi-channel post amplifier used. We expect to improve this somewhat by the use of our 1 kHz filterbank system. It is interesting to compare this frequency with the values predicted by Goss³ in 1966, $3,030 \pm 50$ MHz, and by Baird and Bredohl⁴ in 1971, $3,374 \pm 20$ MHz.

We have looked for CH in one strong OH infrared object, NML Cyg. There is evidence of a faint emission, but our results are not conclusive at this point. Should the results ultimately prove to be negative, one is tempted to believe that CH is very faint or absent in objects which seem to lack formaldehyde. Thus it is not surprising that CH is quite weak in Orion A, which is known also to be weak in formaldehyde.

To illustrate the discussion we have depicted in Figs 1 to 7 the new CH spectra together with OH and H₂CO spectra obtained with the Onsala rutil travelling wave maser radiometers^{5,6} in the following typical sources: W12, Lynds cloud L134, Heiles cloud 2, Cas A, W43N, DR21, and W51. In the figures RC and LC denote OH spectra determined with right and left hand circular polarisation filters, respectively. The OH and H₂CO spectra have been plotted at a resolution of 10 kHz to facilitate comparison with the CH profiles. Note that the direction of positive antenna temperature, as indicated by the arrows, is opposite for CH compared to OH and H₂CO.

The pertinent data from all our observations, excluding NML Cyg, are summarised in Table 1.

We acknowledge help from Dr E. Kollberg and Mr C.-O.

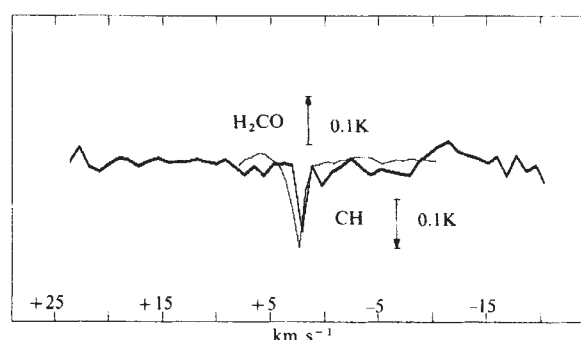


Fig. 2 Lynds cloud L134, CH (—) and H₂CO (—) ground state spectra.

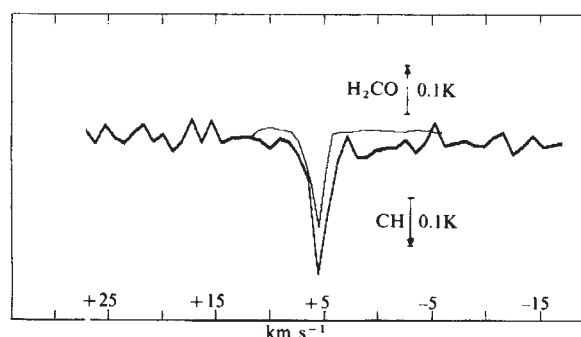


Fig. 3 Heiles cloud 2, CH (—) and H₂CO (—) ground state spectra.

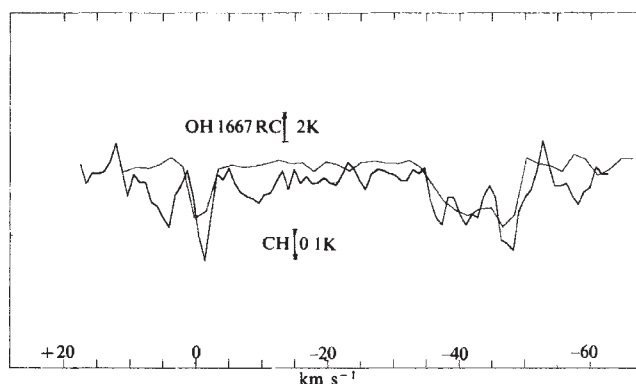


Fig. 4 Cas A, CH (—) and OH 1667 RC (—) spectra.

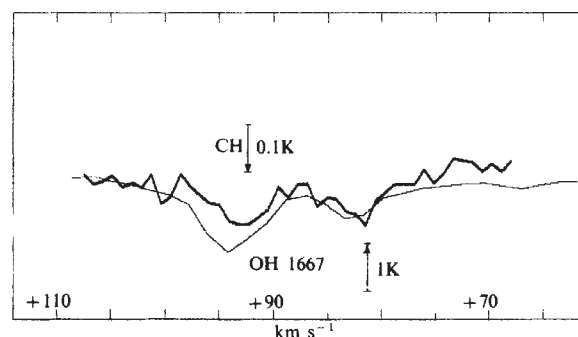


Fig. 5 W43N, CH (—) and OH 1667 (RC+LC)/2 (—) spectra.

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Note added November 19. After these observations were concluded, we also succeeded in detecting both the upper and the lower satellite lines of the ground state Λ doublet of CH. Their frequencies have been found to be $3,349.185 \pm 0.010$ MHz and $3,263.788 \pm 0.010$ MHz. Like the main lines the satellite lines have so far been seen in emission only, even in the direction of the radio source Cas A—a notable circumstance. Although

Table 1 Properties of CH Emission (Onsala Space Observatory)

Object	α_{1950} (h min s)	δ_{1950} (° ' ")	CH velocity of max. feature (km s ⁻¹)	CH, approx. antenna temp. (K)
W3OH	02 23 17	61 39 00	-47	0.05
W3 Cont	02 21 55	61 52 00	-40	0.07
W12	05 39 12	-01 57 42	+9.5	0.15
Orion A	05 32 47	-05 24 21	+10	0.08
W31	18 07 34	-19 56 30	-1.5	0.04
W43N	18 45 01	-02 00 03	+92.5	0.09
W44	18 50 48	01 11 07	+56	0.12
W49	19 07 53	09 01 00	+15.5	0.07
W51	19 21 27	14 24 30	+65	0.10
DR21	20 37 14	42 09 00	-2.5	0.12
W75 B	20 36 50	42 26 58	-2.5	0.06
Heiles 2	04 38 30	25 38 00	+5.5	0.28
Heiles 1B	22 27 55	74 58 00	-4.5	0.08
G84.7-1.0	20 54 23	43 30 00	+0.5	0.15
Lynds				
L134	15 50 54	-04 31 00	+2	0.15
Cas A	23 21 11	58 32 48	-1/-48	0.28/0.24

the intensity of the upper satellite line is roughly 50% of that of the main line (the local thermodynamic equilibrium ratio), the corresponding ratio of the lower satellite to the main line seems to differ from this value in some cases. We classify the transitions as follows:

$$\nu_{10}(F=1 \rightarrow 0+) = 3,349.185 \pm 0.010 \text{ MHz (the upper satellite line),}$$

$$\nu_{11}(F=1 \rightarrow 1+) = 3,335.475 \pm 0.010 \text{ MHz (the main line, } \Delta F=0)$$

and

$$\nu_{01}(F=0 \rightarrow 1+) = 3,263.788 \pm 0.010 \text{ MHz (the lower satellite line).}$$

The ground state $^2\Pi_{1/2}$ $J=1/2$, Λ doublet of CH is a triplet since the ν_{00} -transition is 'forbidden'. In this context it should be mentioned that the four lines of the $^2\Pi_{1/2}$, $J=1/2$ ground state OD Λ -doublet lie in the range 3,090 to 3,115 MHz, that is, well below the CH frequencies.

It is interesting to compare the magnetic hyperfine splittings of the $^2\Pi_{1/2}$, $J=1/2$ state of CH with those of OH, as shown in Table 2. Since none of the CH lines have so far been seen in absorption, irrespective of the nature of the source where they have been found, one is led to believe that all transitions are masing weakly. It could, therefore, very well be that ground state CH is formed totally inverted. This might have important consequences for our understanding of the CH chemical reactions and their rates in the interstellar medium.

Table 2 Comparison of Hyperfine Splittings

Hyperfine splitting	CH	OH
– Parity level (MHz)	71.687	90.414
+ Parity level (MHz)	13.710	14.906

Figure 8 shows, as a typical example, the good correspondence between the ν_{10} , ν_{11} and ν_{01} spectra as seen in the direction of W12. The upper satellite and the main line are quite similar in shape. The lower satellite spectrum, on the other

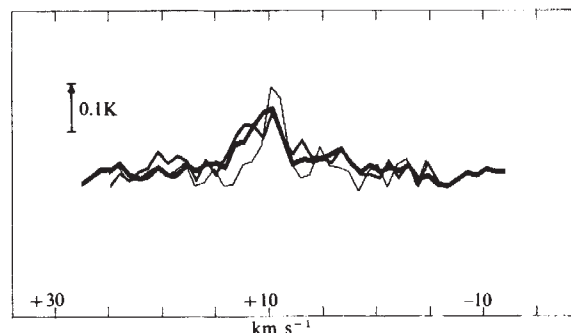


Fig. 8 Emission spectra of the ν_{11} (heavy line), ν_{10} (medium), and ν_{01} (light) lines of the CH ground state Λ doublet, as seen in the direction of W12.

hand, is narrower and has a larger amplitude. Evidently the latter is more strongly masing than the other lines. The same phenomenon is also apparent in the -48 km s^{-1} feature in the direction of Cas A, whereas the ratios between each of the satellite lines and the main line are approximately equal to 0.5 in G84.7–1.0 and Heiles 2. Longer integration times are needed to determine these ratios, as well as the rest frequencies, more accurately. Details of the interstellar satellite line spectra of CH will appear in ref. 7.

We thank Mr Christer Andersson who took an active part in the rapid discovery of the satellite lines, and especially Dr T. Cato who assisted us in the computation of the hyperfine splitting of the ground state doublet.

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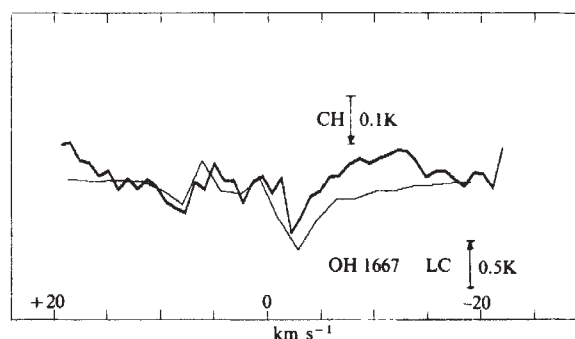


Fig. 6 DR 21, CH (—) and OH 1667 LC (---) spectra.

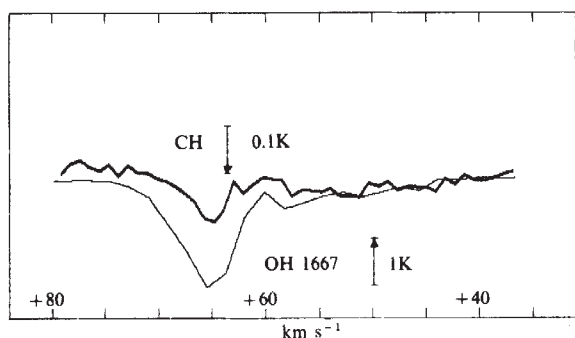


Fig. 7 W51, CH (—) and OH 1667 (RC+LC)/2 (---) spectra.

TiO₂ and a Possible Guide to Past Oceanic Spreading Rates

THE work reported here shows that there is some sort of systematic relationship between the titanium content of present-day ocean-floor basalts and spreading rate. This simple geochemical indicator can be applied to estimate ancient spreading rates using basalts and spilites from ophiolite complexes.

Titanium, expressed as TiO₂, was chosen because it satisfied the following conditions: a reasonable quantity of available data; a good degree of variation between fast and slow spreading ridges; stability under conditions of weathering and zeolite or greenschist facies metamorphism encountered in altered lavas from most ophiolite complexes¹. The data were selected from published major² and trace^{3,4} element analyses of ocean-floor basalts. Picritic lavas containing cumulus crystals of olivine were eliminated as were the rare silicic differentiates and rocks collected from, or near to,

oceanic islands. Samples from locations that were not known with sufficient accuracy for the spreading rate to be determined were also ignored. Samples from the equatorial Atlantic, for instance, were rejected because they may have been generated at 'leaky' transform faults. In all, 171 TiO_2 analyses were used, representing almost all suitable analyses so far published.

Error in spreading rates is difficult to determine. Most rates are averages over the time interval of magnetic anomalies (several million years) whereas basalt compositions may reflect the rates during the times the magmas they represent were generated. The most recent published sources of rates were used here⁵⁻⁷. The location, spreading rate and mean TiO_2 contents for the samples are listed in Table 1. Also in Table 1 are the standard deviations about each mean which give some idea of the spread of values at each locality. The means and standard deviations were both calculated on the assumption of log-normal distribution which has been shown to be most appropriate to this kind of system⁸.

Figure 1 shows the result of plotting mean TiO_2 values against spreading rate on logarithmic axes. A linear regression line and a correlation coefficient were calculated from points based on more than a single sample. The correlation coefficient of 0.7784 for twelve degrees of freedom is significant at around the 0.001 level. Samples from location C5 have been altered and their spreading rate is uncertain. If this location is omitted, a very similar line is obtained, with a correlation coefficient over 0.8. The level of significance suggests that this logarithmic relationship between TiO_2 and spreading rate is real and not due to chance.

Table 1 Summary of Data Used to Relate TiO_2 and Spreading Half Rate

Location	No. of analyses	Spreading half rate	Mean TiO_2	Standard deviation bars (± 1 s.d.) for mean TiO_2
A1 Atlantic 65°–50° N ⁵	13	1.2	1.06	0.99–1.14
A2 Atlantic 50°–35° N ⁵	20	1.3	1.34	1.19–1.52
A3 Atlantic 35°–20° N ⁵	51	1.5	1.51	1.32–1.72
A4 Atlantic 20°–5° N ⁵	1	1.6	1.46	—
A5 Atlantic 41° N 14° W ⁵	4	2.5	1.61	1.19–2.17
PR Palmer Ridge ⁵	8	1.0	1.07	0.90–1.27
JF Juan de Fuca Ridge ⁷	9	2.9	1.78	1.50–2.11
E1 East Pacific Rise ⁷	2	6.7	1.74	1.22–2.49
E2 East Pacific Ridge ⁷	1	8.2	2.10	—
AF Alula-Fartak Trench ⁶	23	1.0	1.41	1.22–1.63
GA Gulf of Aden ⁶	7	1.0	1.02	0.88–1.17
C1 Carlsberg Ridge ⁶	8	1.3	1.46	1.32–1.61
C2 Carlsberg Ridge ⁶	3	1.4	1.42	1.24–1.63
C3 Carlsberg Ridge ⁶	4	2.3	1.38	1.30–1.47
C4 C Indian Ocean Ridge ⁶	10	3.0	1.42	1.24–1.64
C5 C Indian Ocean Ridge (non-axial) ⁶	7	6.5	1.91	1.80–2.02

The means and standard deviations for TiO_2 were calculated on the basis of log-normal distribution.

The explanation of the correlation presumably lies in the variables usually used to explain trace element contents of magmas: depth of melting and liquid segregation, mineralogy and chemical composition of the mantle, degree of partial melting, wall-rock reaction, and crystal fractionation on ascent to the surface. At this stage more information seems to be needed in order to decide how these variables differ in fast and in slow spreading ridges.

Application of the results to basalts and spilites from Phanerozoic ophiolite complexes first requires proof that the lavas in question were erupted at mid-ocean ridges and not in volcanic arc, continental or ocean island situations. Methods have been proposed to do this using Ti, Zr, Y and Sr^{3,4,9} and rare earths^{10,11}. We have chosen the lower pillow lavas of the Troodos Massif of Cyprus as an example of an application of the method since the geological structure, the

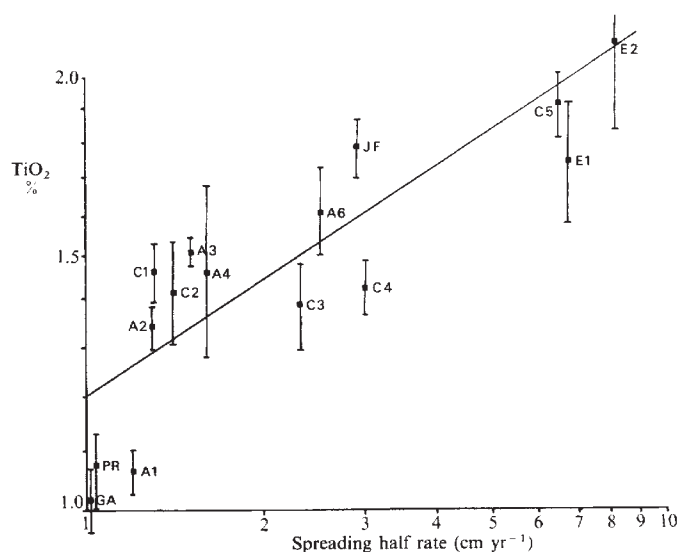


Fig. 1 Logarithmic plot of mean TiO_2 against spreading half rate for the localities in Table 1. The bars show the standard errors ($\sigma/\sqrt{N}$) of each mean based on a standard deviation, σ , of 0.06. $r=0.7784$ significant at 0.001 level.

geophysical properties and the metamorphism of the Massif all closely resemble that inferred for oceanic crust^{12,13}. Chemical evidence suggests ocean floor⁹ and volcanic arc¹⁴ affinities, but the geological evidence for a spreading origin is very strong indeed.

The mean TiO_2 for the Troodos samples was calculated on a log-normal basis as 1.00. Plotted on Fig. 1 this shows a slow spreading half rate of 0.5 to 1.2 cm yr^{-1} . Some independent information is available in this case. The rocks were erupted over a distance of 150 km, measured perpendicular to the spreading axis, during a period of normal magnetic polarity lasting almost 30 m.y. This gives a minimum half rate of 0.5 cm yr^{-1} which does not conflict with the result obtained here. The result also fits in with the interpretation of Moores and Vine¹² of a slow spreading rate based on the abundant presence of chilled margins in the Troodos dyke swarm.

At present this method can only be used with confidence to distinguish fast and slow spreading ridges. With the use of more elements, data from a wider range of locations, more precise evaluation of spreading rates and a more detailed understanding of the petrogenetic variables involved, it may be possible to improve the spreading rate estimates considerably.

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High Resolution Laser Strainmeter

A NEW type of laser strainmeter is now operating in a disused railway tunnel at Queensbury, Yorkshire¹. It uses two helium-neon lasers operating at 633 nm, and has extremely high resolution and linearity. The frequency of one laser, called the slave laser, is locked to the length of a Michelson interferometer attached to the ground. The other laser, used as a reference standard, is stabilised by saturated absorption in iodine vapour²⁻⁵. A similar system has been reported by Levine and Hall⁶ using helium-neon lasers operating at the infrared 3.39 μm wavelength. For practical reasons it is easier to use visible light.

The evacuated Michelson interferometer has a long arm of 55 m, a short arm of 10 cm, and is illuminated by the slave laser. The mechanical and optical details of the interferometer have been described^{7,8}. The slave laser frequency is locked to the length of the interferometer by holding one fringe at the output of the interferometer through the application of a correction voltage to the piezoelectric transducer on which one laser mirror is mounted. The relationship between the frequency ν of the laser and the strain ϵ in the Michelson interferometer is

$$\nu = \nu_0(1/\epsilon) = \nu_0(1 - \epsilon + \epsilon^2 - \epsilon^3 + \dots)$$

where ν_0 is the laser frequency when the strain is zero.

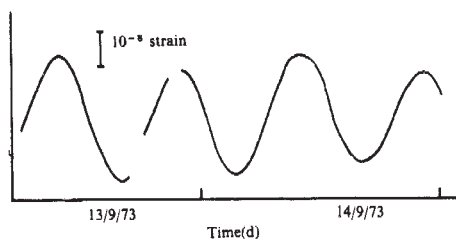


Fig. 1 Earth tides recorded by the laser strainmeter using an iodine absorption stabilised laser.

The light output from the slave laser is optically mixed with light from a frequency-stabilised reference laser to give a beat frequency equal to the difference in frequency between the two lasers. This is recorded digitally and on an analogue chart recorder. In the recording system the beat frequency is divided by 10^4 , and then enters a frequency counter which counts for 100 s every 2 min. Timing is provided by a crystal clock, and the least count corresponds to 2×10^{-13} strain. The resolution could clearly be increased to 2×10^{-17} strain, without altering the counting period, by omitting the divider and feeding the beat frequency directly into the counter. Increasing the length of the interferometer long arm would not change the resolution, although it would reduce the effect of errors introduced by poor coupling of the strainmeter mounts to the ground. Either of these changes could easily be made if required.

The beat frequency is constrained to remain between 10 and 90 MHz, which corresponds to a strain range of approximately

1.7×10^{-7} . When the beat frequency reaches either end of the range the slave laser frequency is stepped to bring the beat frequency back to the middle of the range. This extends the measurable range of strain even up to values which would mechanically damage the instrument. Theoretically, then, the relationship between laser frequency and strain is linear to less than 3×10^{-14} strain. This corresponds to a percentage non-linearity of $1.5 \times 10^{-5}\%$, which is much superior to that provided by any analogue system. In our instrument the linearity is, in practice, limited by the finite gain round the servo loop locking the slave laser frequency to the interferometer length. In principle this non-linearity could be reduced below the theoretical level.

The reference laser used in the system is one stabilised by saturated absorption in iodine vapour. Its frequency stability is better than 8×10^{-12} for the 100 s averaging time used in the beat frequency measurement^{4,5}.

A tidal record for two days is shown in Fig. 1 and there seems to be a relatively small amount of drift. Figure 2 shows a section of the record at high resolution. The record contains frequencies characteristic of microseisms at a reasonable amplitude.

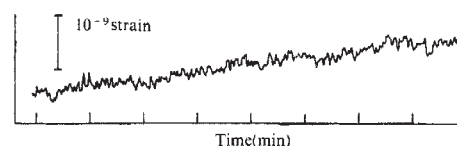


Fig. 2 Record at high resolution using the iodine absorption stabilised laser. Traced from the original chart recording.

This laser strainmeter will be used to study Earth tides and free oscillations of the Earth. The important features of the instrument are the high digital resolution and its exceedingly good linearity. These are very important when studying small signals in the presence of large signals of different frequencies. For example, the cusping of tidal peaks in frequency spectra^{1,9-11} may be due to real Earth effects, but it could equally be attributed to non-linearities in tide-measuring instruments.

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Late Aftershocks, Tectonic Stress and Dilatancy

THE focal mechanisms of micro-aftershocks occurring 3.5 yr after the Inangahua (New Zealand) earthquake of May 23, 1968 (magnitude 7.1), differ drastically from those of the main shock and early aftershocks and are in apparent conflict with the known tectonic stress. This anomaly calls for an explanation as microearthquakes are widely regarded as reliable indicators of tectonic stress. A possible explanation is suggested by the dilatancy hypothesis, which has recently received much attention as a promising basis for earthquake prediction¹⁻⁴.

The usual type of aftershocks, in which the mechanism is like that of the main shock, has already been linked with the flow of pore fluid within the main source region⁵. In the occasional earthquake that involves overshoot, the subsequent deformation in the reverse sense seems to occur by stable sliding without aftershocks⁶. The change in aftershock mechanism observed at Inangahua could possibly have resulted from a delayed outflow of pore fluid from the main source region into the surroundings.

The Inangahua earthquake and early aftershocks have been described by Adams and Lowry⁷. The main shock had epicentral coordinates 41.77° S, 172.01° E, with depth in the upper crust (assigned depth 12 km). Through a 40 d period following the main shock, 809 aftershocks were located including twelve of magnitude 5 or greater. The epicentres were nearly all situated within an elliptical area about 45 km by 25 km. Location was aided by readings from two temporary seismographs which were installed in the vicinity after the main shock. The nearer of these was used for depth control; computed depths ranged from 5 km to 19 km.

The nature of the tectonic stress in the region is known from surface faulting both in the 1929 Murchison earthquake and the Inangahua earthquake⁸ and also from first motion studies^{7,9}. Fig. 1a shows a more extensive compilation of first motion data for some well observed and widely distributed early aftershocks in the Inangahua sequence, together with a replotting of published data⁷ for the main shock. The nodal plane which strikes N 19° E and dips 44° E has an orientation close to that of the known faults in the region; the movement on it is dominantly thrust with a small left-lateral component.

A very different type of faulting was consistently indicated by aftershocks observed about 3.6 yr after the main shock. These were recorded over the period January 4 to 19, 1972, in the course of a regional microearthquake survey which included the area around Inangahua. By means of four portable recorders, numerous earthquakes with magnitudes up to $M_L = 3.1$ were found to be concentrated within an elliptical area situated similarly to the area of the early aftershocks but with the somewhat reduced dimensions 40 km by 15 km. This continued occurrence of aftershocks is to be expected for an earthquake of the magnitude of the Inangahua main shock. Computed depths ranged from 1 km to 20 km, not significantly different from those of the early aftershocks. A composite plot of the first motions of all well observed shocks in this late period is shown in Fig. 1b. These shocks were also well distributed through the aftershock volume. One of the nodal planes again has a strike (N 27° E) close to that of the surface faulting, but the dip is 78° NW. Choosing this as the most likely fault plane one finds that normal faulting was dominant, again with a small left-lateral component.

Between the early and late aftershocks, the fault movement has thus changed from thrust to normal and the dip of the fault planes has swung through about 60°. As the internal consistency of each first motion plot is rather high, it seems that at each stage the seismogenic condition of the aftershock volume was substantially uniform. Some positive explanation is needed for the drastic change in mechanism. In a region where the tectonic stress is compressive, it is difficult to see how pervasive normal faulting can set in, apart from recovery after

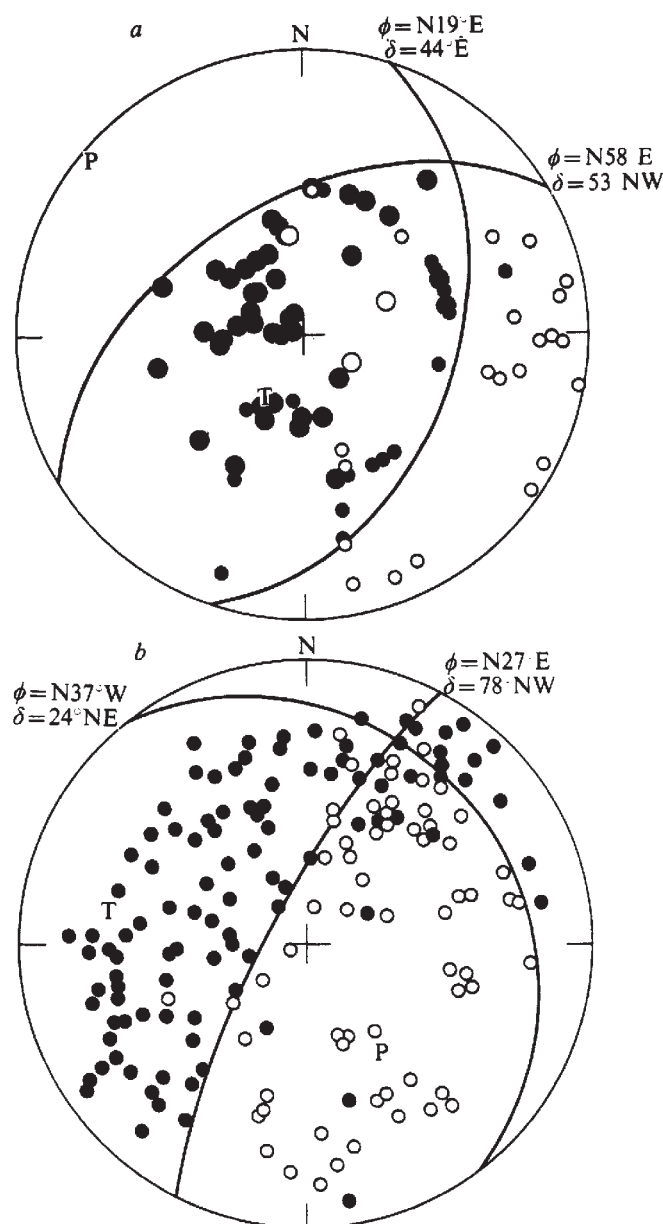


Fig. 1 Composite focal mechanism diagrams of the Inangahua earthquake sequence on equal-area stereographic projections of the lower focal hemisphere. Each nodal plane is specified by a strike ϕ and a dip δ . Idealised axes of compressive and tensional stress are indicated by P and T respectively. a, Main shock (large circles) and early aftershocks (small circles); b, late aftershocks. ●, Compressions; ○, dilatations.

overshoot, unless the tectonic stress is reduced to a low level and material is somehow removed at depth.

An earthquake and its aftershocks serve in general, of course, to reduce the tectonic stress, and there is no great difficulty in supposing a low stress level to have been reached throughout the source region at the time of the late aftershocks. In most shallow earthquakes, as is well known, there is incomplete stress release. Fitch and Scholz⁶ have characterised the motion in this case as overdamped, and the motion when overshoot occurs as underdamped. In these terms we propose that the motion in the main Inangahua earthquake was slightly overdamped. As for the removal of material at depth in the period following an earthquake, this would often be a consequence of pre-earthquake dilatancy.

On the dilatancy hypothesis there is an inflow of pore fluid to the source region before an earthquake. One effect of the earthquake itself, then, is to reduce the dilatancy spaces and thus increase fluid pressure, and if the level of pressure in the

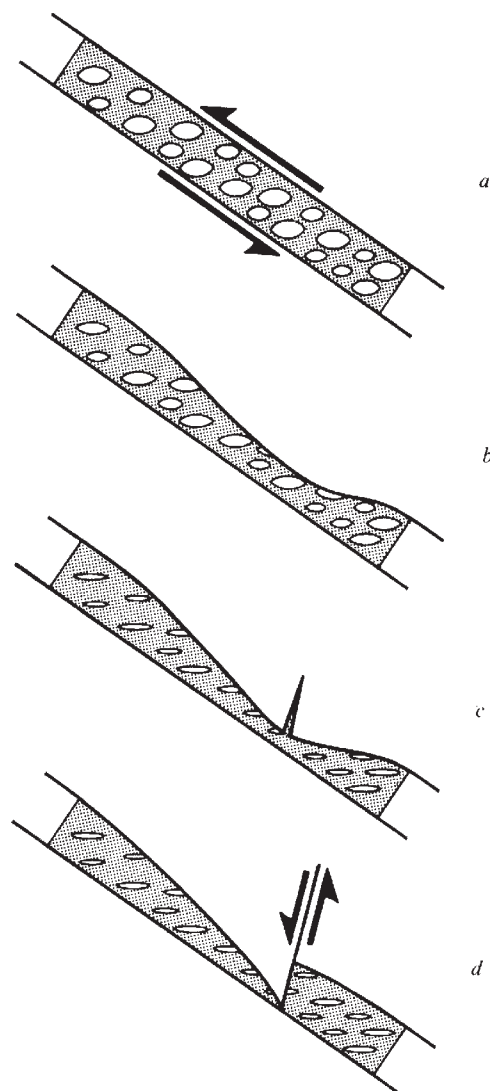


Fig. 2 Schematic representation of change from thrust faulting in main shock and early aftershocks to normal faulting in late aftershocks, as a delayed effect of pre-earthquake dilatancy. *a*, In a pocket containing a high concentration of dilatancy cracks, thrust faulting reduces the tectonic stress to a low level and raises the fluid pressure to a maximum. *b*, Fluid is forced out of the dilatancy cracks and the overlying rock sags. *c*, Further outflow, further sagging, and hydraulic fracturing. *d*, Normal faulting on a steeply dipping plane.

surrounding region is thereby exceeded fluid will begin to be forced out of the source region again⁴. In the broadest terms the observed normal faulting in late aftershocks might be attributed to subsidence following such an outflow of fluid. This would not, however, explain the change in the orientation of the fault plane. Unless there is inherent in the rock a second plane of weakness which is especially favourable to normal faulting, one might expect at first glance that slip should occur on the same fractures that were involved in the main shock and early aftershocks, as in the case of recovery after overshoot. The following simple considerations suggest differently, however, if one invokes the dilatancy concept.

Presumably the dilatancy cracks would be concentrated to some extent in sheet-like pockets along the fault surfaces on which movement occurred in the main shock and early aftershocks. Such a pocket is depicted schematically in Fig. 2; it could be small or extensive. Immediately after the main faulting the fluid would be at maximum pressure and would provide maximum support for the overlying rock (Fig. 2*a*). If the pressure exceeded the ambient value and outflow subsequently occurred, this support would be reduced and the overlying

rock would tend to sag (Fig. 2*b*). Hydraulic fracturing would then tend to develop at points of greatest curvature (Fig. 2*c*), with consequent normal faulting (Fig. 2*d*). The strike of the normal fault would be about the same as for the main faulting, but the dip would be in the opposite azimuth, and steep. This is as observed. We can thus account for the large change in the orientation of the fault plane without introducing the *ad hoc* postulate of a second and conveniently oriented plane of inherent weakness in the rock.

The precursory effects associated with dilatancy, according to Scholz *et al.*⁴, occupy an interval of time which is related to the magnitude of the consequent earthquake. This is the time between the rapid dilation of cracks and the eventual raising of fluid pressure to fracture level by inflow; it is a function of the pressure change, the ground permeability, and the volume of the source region. For the Inangahua earthquake ($M=7.1$) the time would have been about 7.5 yr. Since a roughly similar time should be required by the outflow process it is satisfactory that the observed effect was present after 3.6 yr. The small tectonic stress evidenced by the left-lateral component could have been stress that was left unrelieved by the main shock and early after-shocks, or stress beginning to build up again for the next cycle.

This mechanism for generating microearthquakes as a result of fluid outflow would have no counterpart during the inflow stage. At that time, with the region under compression, the rock would be progressively strengthened as dilatancy reduced the pore pressure. In a region of compressive tectonic stress and, apart from overshoot, it is only late in the stage of fluid outflow that overall conditions would be in any way favourable to normal faulting.

The contrast in focal mechanism between the early and late earthquakes of the Inangahua sequence can thus be interpreted as an effect of dilatancy. This proposal is susceptible to test by the observation of late aftershocks following other main shocks that are due to thrust faulting. If the motion is slightly overdamped and is followed by an outflow of pore fluid, the mechanism of late aftershocks will be more or less strongly influenced by gravity. In view of the long time constant that is attributed to the migration of pore fluid it is not surprising that such an effect should have escaped notice hitherto.

The observations reported here show that a stress orientation indicated by microearthquakes occurring over a substantial area may be very different from that of the tectonic stress. This may be an isolated anomaly with some *ad hoc* explanation. If the dilatancy hypothesis is valid, however, and applicable to events such as that at Inangahua, the explanation proposed above may have some generality.

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Interstadial Site on the Island of Lewis, Scotland

A THIN layer of organic detritus found beneath glacial till in north Lewis has been radiocarbon dated to $27,333 \pm 240$ BP. This dating is an important contribution to an appreciation of British glacial chronology. The significance of the organic bed is enhanced by its yielding sufficient pollen for analysis.

The tabular surface of the Tolsta Head peninsula in north Lewis (Fig. 1) is covered with patches of glacial till. The surface is everywhere mantled with peat. In a shallow depression on the south side of the headland a freshly-eroded cliff section at NB 5572 4682 showed the till resting on laminated silt and organic lake detritus over a distance of 17 m. The stratigraphy at the point of sampling is shown in Fig. 2. On the basis of the interbanding of the finely comminuted rootlets with silt, the organic deposit was classified as lake detritus,

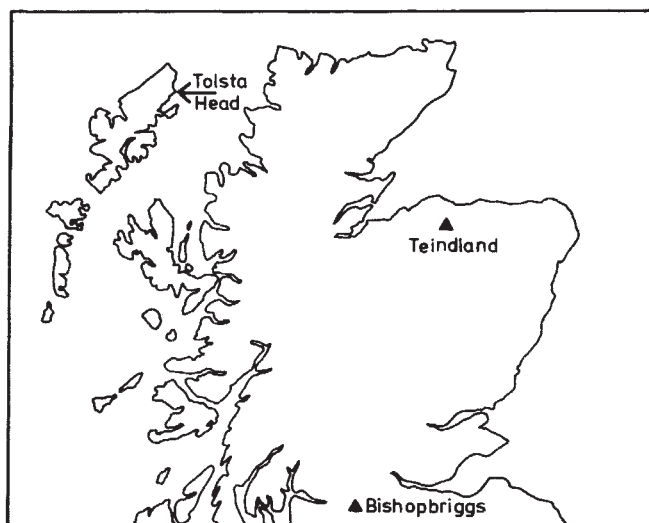


Fig. 1 Site location.



Fig. 2 Organic layer at Tolsta Head.

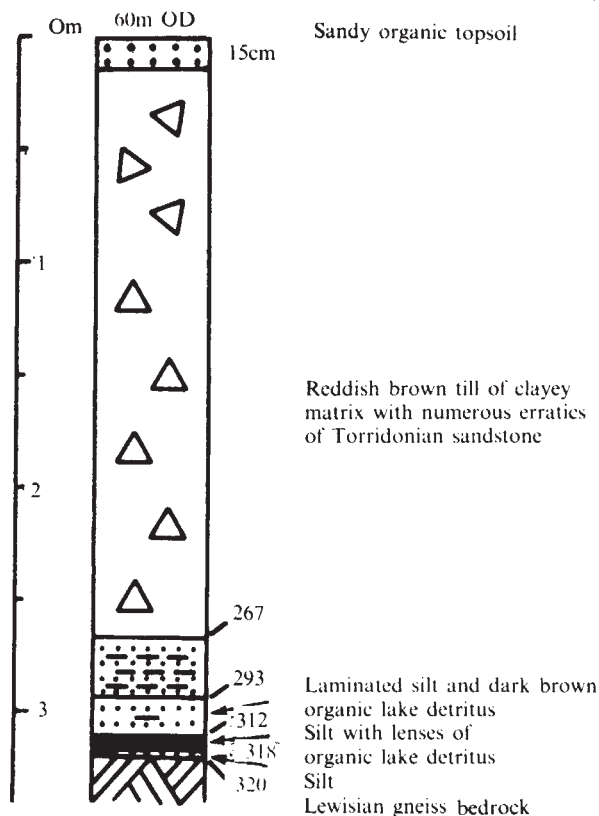


Fig. 3 Stratigraphic column.

which probably accumulated in a temporary lake basin. The bedrock at the site slopes slightly towards the NE. The stones in the glacial till, however, show a preferred SE orientation and dip. Therefore the glacial deposit is unlikely to be of soliflucted origin.

A block from the topmost 15 cm of the laminated silt and lake detritus was collected and submitted for radiocarbon dating to the Scottish Universities Research and Reactor Centre, East Kilbride. The organic material provided an age of $27,333 \pm 240$ BP (SSR-87). The date is based on the Libby half-life of $5,570 \pm 30$ yr. There was no evidence of modern contaminants. The new date is remarkably close to two dates on the Scottish mainland. A buried soil horizon underlying till and outwash gravel at Teindland (Fig. 1) was dated at $28,140$ BP¹. The other is a date of $27,550$ BP for a water-worn bone of a woolly rhinoceros, which was found incorporated in fluvio-glacial sands and gravel at Bishopbriggs² (Fig. 1). Sissons³ has suggested, because the site at Bishopbriggs is not far from the central part of the former Scottish ice sheet, that the whole of Scotland may have been ice free at that time. Similar dates to those from Scotland have been recorded in the English Midlands⁴, the Netherlands and Austria⁵.

Treatment of the deposit with hydrofluoric acid produced sufficient pollen for analysis. The pollen was in a good state of preservation and permitted counts of between 300 and 500 grains per level. The pollen and spore types in the diagram (Fig. 4) are expressed as a percentage of the land pollen sum. The high frequencies of grass pollen and light-demanding herbaceous species such as *Calluna vulgaris*, Compositae and Ranunculaceae indicate an open vegetal landscape. This is further suggested by the presence of juniper, which has a preference for open vegetation⁶. The willows may have occupied damper soils adjacent to a sedge-fringed lake, or perhaps formed a more extensive shrub element, interspersed with juniper over the surrounding area. The isolated grains of *Pinus* and *Picea* must almost certainly have arrived by long-distance wind transport.

The pollen record provides an indication of the climate of

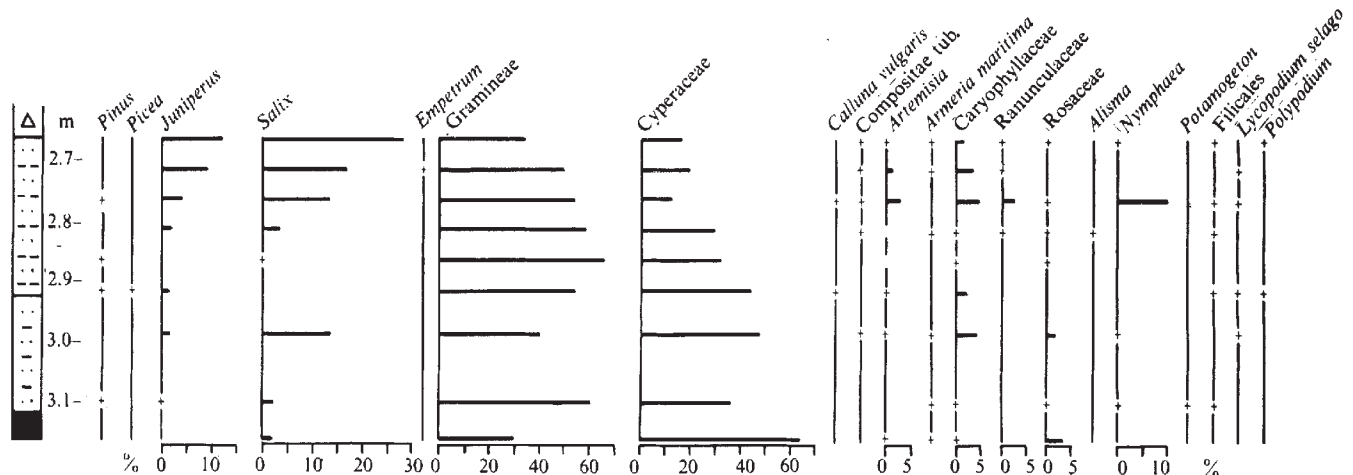


Fig. 4 Pollen diagram from Tolsta Head. +, Indicates less than 2% of sum of land pollen. Note the differing scales for Gramineae and Cyperaceae. Stratigraphic symbols as in Fig. 3.

the time. The taxa present have been found in late-glacial deposits from Britain^{7,8} and mainland Europe⁹. This observation and the lack of tree pollen do not, however, necessarily indicate a severe stadial climate. *Nymphaea* is a genus recorded in late-glacial deposits but is favoured by more temperate conditions¹⁰. *Armeria maritima* is intolerant of extreme cold and is characteristic of areas with an oceanic climatic regime¹¹. The pollen spectra are not inconsistent with the cool maritime climate of Lewis. The increased representation of the warmth-loving juniper¹² in the upper section of the diagram might be considered anomalous before the impending glacial advance responsible for the overlying till. Although the species possesses a wide ecological amplitude¹³, a possible interpretation is that the deposit has been truncated by glacial erosion. The profile may therefore be incomplete.

The significance of the dated deposit lies in its stratigraphic context. The overlying glacial till contains numerous erratics of Torridonian sandstone, a rock type alien to the lithological assemblages of the Outer Hebrides. The abundance of these erratics in the till suggests deposition by an ice mass which had moved over at least part of the Minch. This suggestion is supported by two other lines of evidence. Till-fabric analysis on the site revealed a predominant stone orientation of 50° W of N to 50° E of S and dip towards the SE. Also, till with shell fragments is exposed in a coastal section 1.2 km west of the site¹⁴. Other glacial deposits containing Torridonian sandstone erratics and broken shells, but of a more complex nature, have been found on the Eye peninsula and at the northern tip of Lewis^{15,16}.

The organic layer has thus produced a third date in support of interstadial conditions in Scotland at 27,000 to 28,000 BP. Pollen analysis gives an indication of the vegetation and climate at this time. The stratigraphic sequence at the site provides evidence for the presence of glacier ice at Tolsta Head after about 27,330 BP.

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Multiple Discriminant Screening Procedure for Test Ban Verification

A DECADE of research has led to a well defined capability for the seismological method of verification of compliance with a ban on underground nuclear explosions. Whatever political form the treaty will eventually have, it is generally agreed that decisions concerning violations will be national decisions. Recent presentations to the Conference of the Committee on Disarmament in Geneva¹⁻⁴ lead us to consider in some detail utilisation of the seismological discrimination experience in a national operational verification procedure. The thrust of the sample operational procedure to be described depends on an acceptance of the fact that detailed consideration of clandestine explosions, or evasion tactics in general, is futile until all natural seismic activity can be identified down to the required seismic magnitude or equivalent explosion yield. Thus, the operational procedure becomes an earthquake screening process that can be tested in simulation in advance of the Test Ban Treaty.

The seismic region chosen for a simulation is a major portion of Eurasia (Fig. 1) and the monitoring nation can be assumed to be Canada, although with the data exchange procedures inherent in the scheme it could be any nation. The seismic events considered are from the Lincoln Laboratory, MIT bulletin for the 29-day 'International Seismic Month' (ISM)⁵, the most comprehensive global seismic bulletin assembled to date on the basis of teleseismic observations; the number of events in each of five regional blocks is shown in Fig. 1.

The total of 291 events, shown in cumulative distribution in Fig. 2a, are subjected to three types of earthquake screening: (a) those with epicentres at sea, (b) those with focal depths greater than the maximum feasible drilling depth, and (c) those that are earthquake-like by one or both of the $M_s:m_0$ and P-wave discrimination criteria. The result of this screening is shown in all combinations as histograms

in Fig. 2b to e. The location screening assumes that nuclear test emplacement is impractical from a location at sea and events are screened as earthquakes if their epicentres are at sea by at least the largest epicentre error ellipse semi-axis. The result is 152 earthquakes at sea (Fig. 2b and c) and 139 events with epicentres on land or within the epicentre error of a land mass (Fig. 2d and e). The focal depth screening assumes that nuclear test emplacement is impossible at depths greater than maximum feasible drilling depths, assumed here to be 10 km. Events are screened as earthquakes if their computed focal depths are greater than the largest hypocentre error ellipsoid semi-axis plus 10 km. The independent result of this screening is 158 deep events (thus earthquakes) shown in Fig. 2b and d and 133 events with depths less than 10 km or with computed depths within the hypocentre error of this depth. (Note that the word 'deep' as used here refers to deeper than 10 km, rather than the more standard usage for earthquake depths.) The combined result is eighty-eight events (30% of the total) in Fig. 2e that remain for further consideration as possible explosions.

To screen these events using discrimination criteria, we have used a formal two-group discrimination analysis⁶. In brief, accumulated experience with the $M_s:m_b$ criterion⁷ and the Yellowknife array P-wave criterion^{8,9} for the Eurasian region for earthquakes and underground explosions are used as design sets to define multi-parameter discrimination functions that optimise the separation between the explosion and earthquake populations. A decision level is chosen in the multi-parameter hyperspace to screen earthquakes with near certainty. For each of the ISM events for which discrimination results are available, the position in hyperspace is computed and an earthquake decision made if the event falls to the earthquake side of the decision level. For the $M_s:m_b$ criterion, which is two-dimensional, this decision level can be presented visually with reference to the standard $M_s:m_b$ plots: the earthquake decision is made for events with $M_s \geq 1.5 m_b - 3.4$. This is intended to be a cautious earthquake decision level and, for example, requires M_s half a magnitude greater than those of the most earthquake-like explosions in the plot of Marshall and Basham⁷.

An equivalent procedure is employed for the P-wave criterion for which the hyperspace is twelve-dimensional. The Yellowknife array P-wave discriminant utilises the shorter duration, impulsive nature of explosion P waves, as measured by seven parameters in the time domain, and the greater relative high frequency content of explosion P waves, as measured by five parameters in the frequency domain, to distinguish them from earthquake P waves. For this

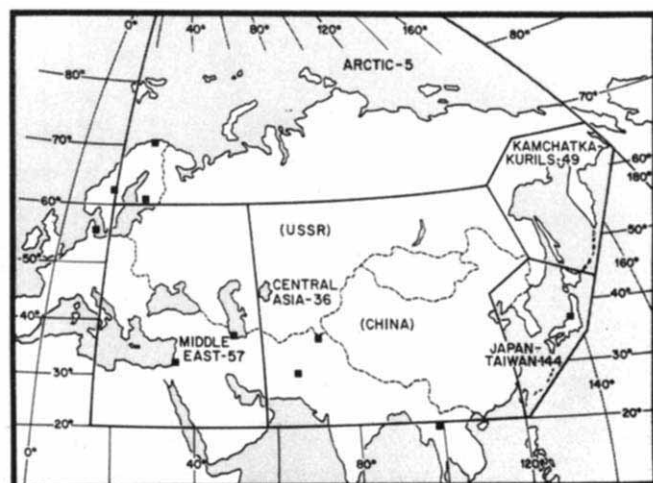


Fig. 1 International Seismic Month seismic event distribution in five Eurasian geographic blocks. Solid symbols show locations of Eurasian seismograph stations used for M_s magnitudes.

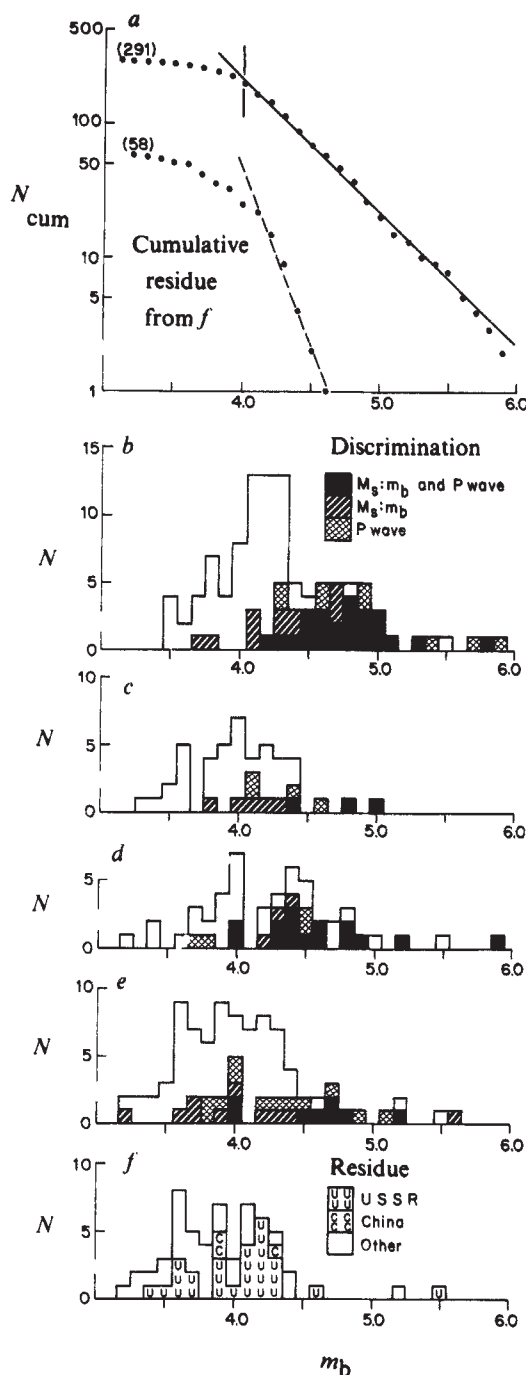


Fig. 2 Eurasian earthquake screening results for the International Seismic Month. a, Total cumulative event distribution illustrating bulletin is 90% cumulative complete at $m_b 4.0$; b, events with epicentres at sea and focal depths greater than 10 km; c, at sea and shallower than 10 km; d, on land and deeper than 10 km; e, on land and shallower than 10 km; f, final screening residual events. Earthquake decisions by discrimination criteria are shown as shading in b to e. Residual events are shown in cumulative distribution in a.

application, the decision level is chosen in a similarly cautious manner with respect to experience with the most earthquake-like explosions in the large population studies (ref. 8 and F. M. A. and H. Israelson, in preparation).

As shown by the histogram shading in Fig. 2b to e, fifty earthquake decisions can be made on the basis of the $M_s:m_b$ and P-wave criteria together, thirty on the basis of the $M_s:m_b$ criterion alone and twenty-nine on the basis of the P-wave criterion alone. All but eight, ten and twelve of these decisions, respectively (those in Fig. 2e), are redundant,

the other events having previously been screened by location or depth. The residue of events that have not been classified as earthquakes to this point in the procedure is shown in Fig. 2f.

It is clear from Fig. 2 that the probability of earthquake decisions on the basis of the discrimination criteria decreases rapidly at magnitude below $m_b 4.5$. In addition, the ISM bulletin becomes incomplete as the magnitudes decrease much below this value: being 90% cumulative complete at $m_b 4.0$, it is 90% incremental complete at about $m_b 4.3$. The threshold of this monitoring experiment has therefore been set on an equivalent level, say $m_b 4.3$, by both the event detection/location capability and the earthquake identification capability. To continue the experiment further in terms of simulated verification would require some assumptions about the goals of the procedure in a treaty situation. For example, if $m_b 4.3$ were the desired threshold capability, then the ten events in Fig. 2f with magnitudes $\geq m_b 4.3$ would be treated exhaustively to attempt to establish their identity. This will not be attempted here, but some general comments are of value. The $m_b 5.5$ residual event was in Eastern Kazakh and is identified as an explosion by all criteria; such a larger explosion at the principal Soviet test site is largely irrelevant to discussions of a treaty situation. Three of these ten events are believed to have overestimated magnitudes; two have observable depth phases that suggest they are deeper than 30 km; we have no additional information for the other four, each at $m_b 4.3$.

Table 1 Projected Annual Numbers of Eurasian Earthquakes

Category	$\geq m_b 4.0$	$\geq m_b 4.5$
(1) Total for Eurasian region considered	2,800	880
(2) USSR land mass epicentres	450	140
(3) China land mass epicentres	140	50
(4) Total screening residue	630	38
(5) USSR residue	315	(13)
(6) China residue	(13)	(0)

In addition to the actual numbers of events available for the 29-day period in the above screening process, the recurrence curve (Fig. 2a) for the events is sufficiently well defined to allow some projections of annual numbers of events that would require treatment by such a procedure. Comparisons of annual projections of these events with equivalent data presented by Evernden⁹ and Anglin¹⁰ demonstrate that the ISM is adequately representative of average Eurasian earthquake rates. As the ISM bulletin is the most comprehensive one to date based on the m_b scale, it probably provides the most accurate available estimates of numbers of land mass earthquakes in the Soviet Union and China in the m_b range ≥ 4.0 . For the annual occurrence rates given in Table 1, the projected 100% occurrence rates for the ISM period are simply multiplied by 365/29.

Some indication of how an operational verification scheme intended to monitor the Soviet Union and China can treat these, say, 600 annual land mass events ≥ 4.0 is available from the 'shallow land mass' residue in Fig. 2f. The residual events, with the $m_b 5.5$ explosion removed, are plotted in cumulative form as an inset in Fig. 2a. As each of the location, depth and discrimination screening criteria is removing events in a magnitude-dependent and region-dependent way, there is no certainty that the residue will have an equivalent recurrence distribution. If, however, the straight line shown is accepted as representative of the residue, some projections can be made, and are listed as categories 4, 5 and 6 in Table 1. An interpretation that can be made of these projected residue events is to assume that the screening procedures outlined here applied in a routine way by the monitoring nation; the residue then represents those events that must be subjected to additional analysis in

order to establish their sources as being earthquakes or explosions.

In summary, it is important to note that the ISM bulletin used for the simulation is increasingly incomplete at magnitudes below $m_b 4.3$, even though its compilation made exhaustive use of the best P-wave detection facilities in existence. Thus, any nation requiring a verification (earthquake screening) capability at about $m_b 4.0$ must first deploy additional facilities to detect and locate the events. If a nation were to monitor the Soviet Union and China with some substantially increased facilities over those that currently exist and concern itself with all events ≥ 4.0 , it must assess about 600 events per year with land mass epicentres. It seems very unlikely that, using teleseismic techniques, discrimination criteria will be devised to screen confidently all of these events as earthquakes. If an explosion is detonated under some evasion scenario whereby either the explosion cannot be assessed as an independent seismic event or it is screened as an earthquake using procedures such as those described above, then the seismological method will not note a violation of the treaty. These are simplified statements of the limitations inherent in the seismological method.

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New Spin Structure in an Amorphous Ferric Gel

AMORPHOUS materials have no preferred crystal axes, but it might be thought that shape or strain could define an anti-ferromagnetic axis in a compound containing a large concentration of magnetic ions with negative nearest-neighbour exchange interactions. We have studied such an amorphous compound, and find an ordered state where spins are coupled with their directions statistically distributed within a very fine particle. This spin structure is quite different from normal antiferromagnetism, although there are some similarities in the magnetic properties.

The material studied was precipitated near freshwater springs on Caton Moor, Lancashire; 97% of the inorganic fraction was a ferric gel with empirical formula $\text{Fe}(\text{OH})_3 \cdot 0.9 \text{H}_2\text{O}$, and

it was mixed with 30% of organic matter and 2% of silicate minerals. No evidence for crystallinity was found by X-ray or electron diffraction, and the material was almost completely dissolved by acidified ammonium oxalate, a treatment which extracts only the non-crystalline fraction¹. Details of the characterisation and magnetic properties of the amorphous gel are to be published. The main features of its magnetic behaviour may be summarised as follows. Below about 10 K it is clear from the existence of a remanence and from the Mössbauer spectrum of Fig. 1a that there is an ordered magnetic structure. The moment obtained by extrapolating the linear portion of the 1.6 K magnetisation curve (above 50 koersted) back to 0 koersted is 8.6 e.m.u. per g of gel. The Mössbauer spectrum becomes a simple quadrupole doublet above 20 K, yet Curie-Weiss paramagnetism is found only above 100 K. The gel is in the form of ultra-fine particles ($\lesssim 100$ Å) and the magnetisation curves, plotted as a function of H/T , superpose between 30 and 80 K. They may be represented by a Langevin function with a spontaneous moment of 7.2 e.m.u. g⁻¹, in satisfactory agreement with the 1.6 K extrapolation, and a moment per particle of 73 μ_B (ref. 2). In other words, the collapse of the Mössbauer hyperfine splitting above 10 K corresponds to the unblocking of the superparamagnetic fine particles, and the true magnetic ordering temperature is higher, about 100 K.

Here we are concerned with the spin structure of these amorphous fine particles. If they were antiferromagnetic (Fig. 2b) the spins of a particle would all lie in a line. The particle's weak moment would arise from the statistical imbalance of up and down spin, and would be parallel to the spins. Alternatively, the weak moment would conceivably result from a slight canting of the sublattices, and would then be almost perpendicular to the spins, as in $\alpha\text{Fe}_2\text{O}_3$. Which configuration, if either, is correct can be decided from Mössbauer spectra in applied fields, shown in Fig. 1. Fields of 10 and 50 koersted were chosen which almost (5.8 e.m.u. g⁻¹) or completely (12.8 e.m.u. g⁻¹) saturate the weak moment. In zero applied field, lines 2 and 5 comprise $34 \pm 2\%$ of the total absorption,



Fig. 2 Possible spin structures for an amorphous compound. a, Ferromagnetic; b, antiferromagnetic; c, sperimagnetic.

as is usual for the random alignment of spins relative to the γ -ray direction found in a multiparticle sample³. When the field is applied parallel to the γ direction the absorption in lines 2 and 5 would be 0% if all the spins were parallel or antiparallel to the field, as expected if a weak moment arising from the first spin configuration was saturated. Also the spectrum should double, or at least show structure, at 460 ± 50 in Fig. 1c as the applied field adds to one sublattice and subtracts from the other. Conversely, if the spins were almost perpendicular to the field, as expected for the second configuration, or if a spin flop had occurred, the absorption in lines 2 and 5 would be 50% of the total. The calculated spin flop field actually is 100 koersted for these particles, much greater than the applied fields. In Fig. 1 it is obvious that neither of the above possibilities is the correct one. The spectrum is hardly changed by the applied field, 33% of the absorption remaining in lines 2 and 5 in 10 koersted, and 29% in 50 koersted. There is no doubling of structure, but only some broadening of the outer lines. In summary, the data are not consistent with any kind of antiferromagnetism in these fine particles, but they show that the spins within a particle are statistically distributed relative to the direction of their resultant moment.

Such a configuration, with the spins fixed relative to each other but possessing no overall preferred direction, is illustrated in Fig. 2c. It is termed 'sperimagnetic' (Greek διασπειρω, I scatter), and although it has only been possible here to demonstrate its existence in ultrafine particles because they have a resultant moment relative to which the other spin directions can be measured, it is probably a fairly common phenomenon in amorphous compounds.

The resultant moment of a particle containing N ions, each of moment m , pointing in random directions is $\sqrt{N}m$. The value of the hyperfine field at 4 K (460 koersted) shows that the ferric moment is only a little less than its ionic value of $5 \mu_B$. From the superparamagnetic magnetisation curves we deduce that each particle contains an average of 635 formula units, and the predicted moment is thus $\sim 126 \mu_B$, rather larger than observed. The difference can, however, be accounted for as follows. The Curie constant gives an unexpectedly small effective moment of $3.68 \mu_B$, but this is also observed in synthetic gels where it is explained by the equilibrium concentration of a ferric dimer, built into the gel structure as it is precipitated⁴. Thirty-eight per cent of the iron is thus coupled rigidly antiparallel to an iron neighbour and will not contribute to the net moment. The predicted particle moment is now $78 \mu_B$, in excellent agreement with the observations.

There are some similarities between sperimagnetism and the magnetism of alloys such as Cu Mn or Au Fe which are known as mictomagnetic alloys or spin glasses. A mictomagnet^{5,6} is also characterised by the freezing of spin orientations at low temperatures without any long range spin order, but within this matrix there are clusters of short range order, which possess giant moments. The magnetisation in a zero field cooled specimen passes through a maximum near the temperature where the remanence goes to zero, and the hysteresis loop is considerably displaced by field cooling. Neither of these effects were observed in our material. The same alloys are known as spin glasses at lower concentrations of the magnetic

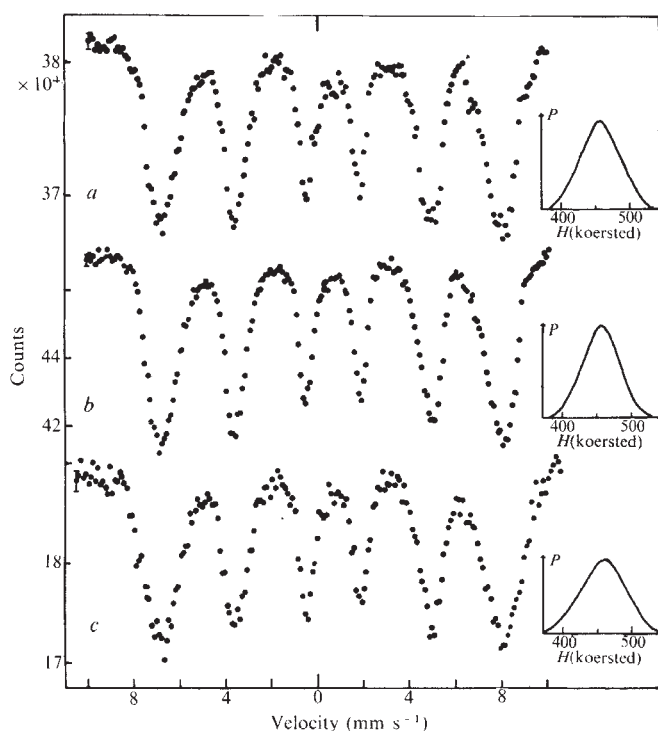


Fig. 1 Mössbauer spectra of amorphous $\text{Fe}(\text{OH})_3 \cdot 0.9 \text{H}_2\text{O}$ at 4.2 K in a field of 0 koersted (a), 10 koersted (b) and 50 koersted (c) applied parallel to the γ direction. The insets show the hyperfine field probability distributions, $P(H)$.

solute atoms. They are characterised by both ferromagnetic and antiferromagnetic coupling, but it has recently been emphasised that any understanding of the microscopic structure of the ordered state is still very unclear⁷, particularly concerning the formation of clusters.

Finally, we consider possible reasons for the random orientations of the spins in a sperimagnet. (The orientations are random in a volume containing $\sim 10^2$ spins; there is nonetheless a net antiparallel correlation in the shell of first neighbours.) A possible explanation might be that the single ion anisotropy is very large for certain cations (though not for Fe^{3+}) in the irregular ligand coordination polyhedron, and the spin direction is thus fixed by the local charge environment. It is more likely, however, that the spin directions are determined by the balance of exchange interactions, such as give rise to the canted spin structures in diamagnetically substituted ferrites⁸ and the disoriented spin structure suggested for the surface of ultrafine $\gamma\text{Fe}_2\text{O}_3$ particles^{9,10}.

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Optical Elastohydrodynamics at Extreme Pressures

THE theoretical predictions of Dowson and Higginson¹ concerning the size of the lubricant film generated in rolling-bearing line contacts have been adequately confirmed over a fairly large range of pressures and for a wide selection of temperatures, speeds and fluids (see, for example, ref. 2). The theory has been extended to include point and elliptical contact conditions³⁻⁵ and various aspects of non-Newtonian fluid behaviour (see, for example, ref. 6). Lately, however, there has been a need to test the predictions at much higher pressures as the loading in modern bearings constantly increases. Since optical elastohydrodynamic techniques^{7,8} have been limited to a pressure of about $7 \times 10^8 \text{ N m}^{-2}$ (100,000 pound inch⁻²) by the use of glass as one lubricated element, this investigation has largely fallen to the X-ray method⁹. This technique has been doubted by some workers although it has generally reaffirmed the data obtained with earlier methods of film thickness measurement. Unfortunately,

recent results¹⁰ with this method differ greatly from theory. Film thicknesses have been found an order of magnitude below predicted values at pressures of $2 \times 10^9 \text{ N m}^{-2}$, and the dependence on load has been generally too great. It is therefore uncertain whether this is a failure of the Dowson and Higginson type of theory or of the X-ray method itself.

The work outlined here concerns an extension of optical elastohydrodynamic film thickness measurements in pure rolling to pressures of over $2.0 \times 10^9 \text{ N m}^{-2}$. This has been achieved using a lubricated sapphire disk, over which rolls a highly polished 1-inch diameter tungsten carbide ball. This replaces the combination of steel on glass used by previous workers⁷ but is otherwise very similar.

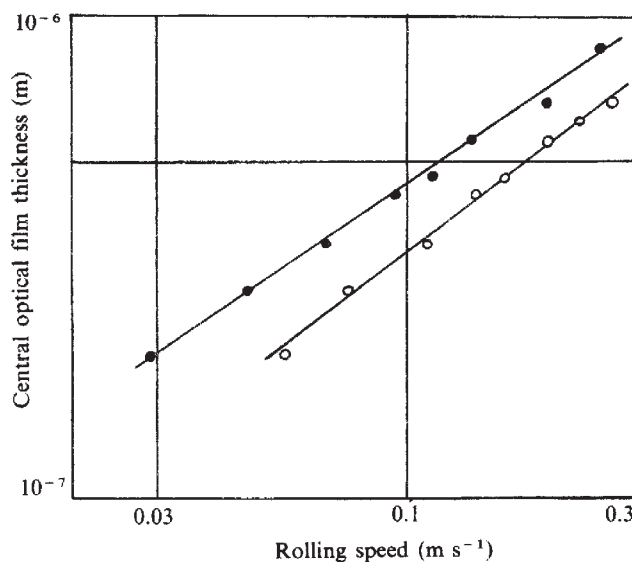


Fig. 1 Typical film thickness/speed results (fluid 3). Maximum Hertz stress: ●, $8.4 \times 10^8 \text{ N m}^{-2}$ (1.2×10^6 pound inch⁻²); ○, $20.7 \times 10^8 \text{ N m}^{-2}$ (3.0×10^6 pound inch⁻²).

Three fluids have been examined: (1) a synthetic paraffin, as used in ref. 10; (2) naphthenic mineral oil base stock; and (3) fluid 2 plus 10% W paraffinic heavy resin. They were tested at nine loads to cover the range of pressures from 0.8 to $2.0 \times 10^9 \text{ N m}^{-2}$. For the time being the temperature used has been ambient. The experimental method was to increase the rolling speed from zero while observing the point contact with chromatic interferometry. At each of at least eight set film thicknesses the rolling speed was measured. The results are displayed graphically as in Fig. 1. The variation of film thickness with load, which is in question, was obtained by drawing lines at chosen rolling speeds and reading off the value of film thickness at the intercept for each load. The results obtained by this method are given in Fig. 2. They show an almost linear variation of film thickness with load over the whole range, according to the relationship

$$h \propto W^{-a}$$

with a as given. Owing to the fact that point contact pressure increases as the cube root of the load, an estimate of error in the pressure data is about $\pm 10\%$ at low loads but better than $\pm 1\%$ at high loads. On the other hand, at high loads there is an unavoidable rise in temperature of the fluid at the contact inlet, which reduces the film thickness and could account for the slight divergence from a straight line in that range. In addition the film thickness quoted is the optical one:

$$\text{optical thickness} = \text{real thickness} \times \text{refractive index}$$

The refractive index is not certain at these conditions since it increases with pressure and falls with temperature but, according to Paul and Cameron¹¹, probably changes less than 5%.

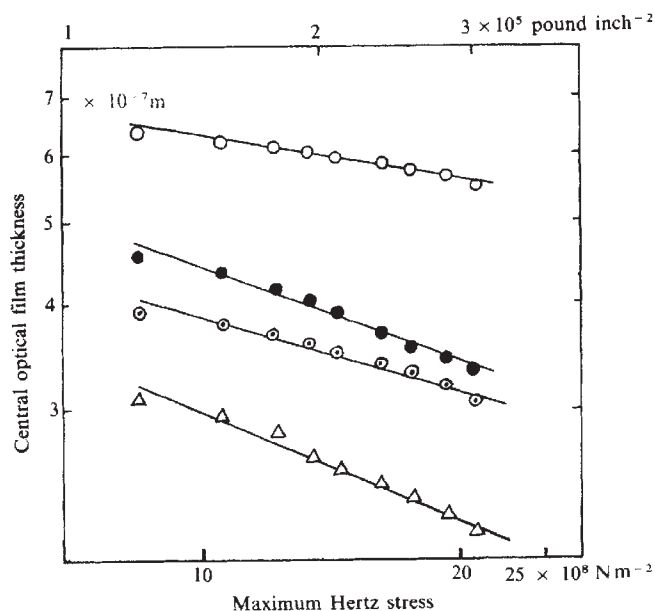


Fig. 2 Variation of film thickness with pressure. $\circ$, Fluid 1, 0.1 m s^{-1} , 28.5° C , $a=0.067$; $\odot$, fluid 1, 0.05 m s^{-1} , 28.5° C , $a=0.100$; $\triangle$, fluid 2, 0.1 m s^{-1} , 22° C , $a=0.144$; $\bullet$, fluid 3, 0.1 m s^{-1} , 22° C , $a=0.125$. Dowson and Higginson (line contact), $a=0.13$; Archard (point contact), $a=0.083$.

In conclusion it may be said that there was no evidence of a marked fall in film thickness for the three fluids beyond what it normally expected. It is difficult to compare against theory since various parameters, such as the pressure-viscosity coefficient, are not known, but there is certainly not the order of magnitude divergence found in ref. 10. There is therefore apparently a large discrepancy between optical measurements and X-ray measurements, even noting the difference in temperature range, film thickness and shear rate and that the former are central film thicknesses whereas the latter are minimum film thicknesses. Certainly film thickness data at these extreme pressures must be regarded with suspicion until a definitive study can be made of the problem.

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Speed of Sound in Bent Tubes and the Design of Wind Instruments

TRUMPETS, bassoons and other long wind instruments are made with bends for compactness. They would function best if each bend behaved acoustically just like a definable segment of straight tube. The custom of the craft is to make each bend a semi-torus of the same bore diameter as the straight tubes that it joins if these are cylindrical, and roughly a semi-torus of bore diameter intermediate between the diameters of the two straight tubes that it joins if these taper. This custom (which is very old) seems to find justification in the argument of Rayleigh¹ who, from the assumption that the velocity potential is always invariant over any section perpendicular to the bore, derived an equation according to which a curved tube is acoustically equivalent to a straight tube of the same cross-sectional area and the same length measured along the central axis. Nederveen³, by assuming the pressure to be always invariant over any cross-section of the bore and calculating the inertial consequences of his assumption without regard for continuity of the air, obtained the different result that a toric segment of bore diameter d , generator circle diameter D and length s measured along the central axis is acoustically equivalent to a cylinder of bore diameter $d\sqrt{a}$ and length s/a , where

$$a = (D\sqrt{2}/d) [1 - (1 - d^2/D^2)^{1/2}]^{1/2}$$

from his equations (37.6) and (37.9).

Now neither Rayleigh's nor Nederveen's assumptions can be quite true. Both imply strictly laminar flow; Rayleigh's directly because if the velocity potential is invariant over a plane there can be no component of velocity in that plane; and Nederveen's indirectly, because if the pressure is invariant over a plane there can be no component of acceleration in that plane. But strictly laminar oscillations in a torus are self-contradictory if inertia and continuity are both taken into account, since the lengths of inside and outside laminae are different. An exact theory seems likely to be very difficult. I therefore experimented with resonators. Each of these (Fig. 1) consisted of two straight tubes of lengths 36 and 72 cm and internal diameter 0.95 cm joined by a semi-torus of bore diameter 0.95 cm (later also smaller bore diameters) and generator circle diameter 1.3 or 4.5 cm. The resonators, with one end closed, were driven from an oscillator and loudspeaker so that there were 3, 9 or 15 quarter wavelengths from end to end. In each such mode of resonance, if the short end is closed the bend lies at a velocity antinode and if the long end is closed it lies at a velocity node. Table 1 shows the resonant frequencies (means and standard errors of means of three measurements).

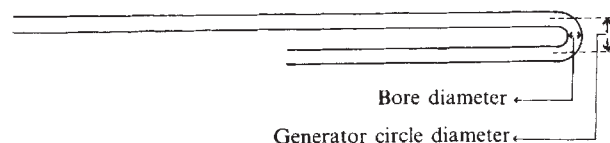


Fig. 1 One of the resonators used in the measurements (not to scale).

For each resonator and mode of resonance, measurements with the short end closed and with the long end closed were alternated. Thus any slow drifts in temperature which may have occurred are unimportant in this comparison. No such precautions were taken between measurements for different modes of resonance; it would therefore be unsound to use Tables 1 and 2 to calculate the effect of frequency on end-corrections.

It can be seen that the semi-torus is equivalent to a shorter straight tube when it is antinodal than when it is nodal. The effective shortening is roughly independent of frequency over

Table 1 Resonant Frequencies (Hz) of Resonators whose Toric Segments had Bore Diameter 0.95 cm and Generator Circle Diameters 1.3 cm or 4.5 cm

Mode of resonance	Generator circle diameter 1.3 cm		Generator circle diameter 4.5 cm	
	Long end closed	Short end closed	Long end closed	Short end closed
Quarter wavelength = 1/3 of tube length	227.8 ± 0.20	229.3 ± 0.13, difference = +1.5	223.9 ± 0.10	224.4 ± 0.13, difference = +0.5
Quarter wavelength = 1/9 of tube length	686.8 ± 0.25	691.1 ± 0.31, difference = +4.3	672.7 ± 0.06	673.9 ± 0.18, difference = +1.2
Quarter wavelength = 1/15 of tube length	1,150.0 ± 0.42	1,155.7 ± 0.32, difference = +4.8	1,128.8 ± 0.24	1,130.6 ± 0.30, difference = +1.8

the range investigated, the slight apparent decrease at high frequencies being probably mainly due to the fact that the torus then occupies an appreciable fraction of a wavelength. It is less for a gentler bend. An intermediate generator circle diameter (not shown in Table 1) gave an intermediate result.

The observed effect is in the same direction as that implied by Nederveen's formula and greater by a factor which varies, for the six examples in Table 1, between 1.2 and 1.8.

In a wind instrument that has to sound high modes of resonance it is undesirable that either the damping or the effective length should depend on the relation of the wave-pattern to any bends that are inserted not for their acoustic virtue but merely for compactness. The damping is presumably generally greater when velocity antinodes occur at bends, where the flow cannot be regular, than when they all occur in straight tube, where it can. The present experiments showed that this influence on damping is small, and almost certainly negligible for musical purposes; the Q at resonance was, for the resonators of Tables 1 and 2 and for another whose toric segment had bore diameter 0.86 cm, always the same within 1%, whichever end of the resonator was closed. The effective length is, on the contrary, influenced by the relation of wave-pattern to bends enough for the influence to be musically important. It can be corrected by joining straight cylindrical tubes with a torus not of the same bore diameter but of slightly less. From the results in Table 1, the perturbation theory of pipes (following Rayleigh²) predicts that for the first of the two resonators a reduction in bore diameter of torus from 0.95 to 0.91 cm should compensate. Experimentally, such a resonator gave the results shown in Table 2 (means and standard errors of means of three measurements).

Table 2 Resonant Frequencies of a Resonator whose Toric Segment had Bore Diameter 0.91 cm and Generator Circle Diameter 1.3 cm

Mode of resonance	Long end closed	Short end closed	Difference
Quarter wavelength			
= 1/3 of tube length	228.2 ± 0.13	228.2 ± 0.06	0.0
= 1/9 of tube length	689.4 ± 0.00	689.4 ± 0.11	0.0
= 1/15 of tube length	1,155.7 ± 0.37	1,155.1 ± 0.37	-0.6
Quarter wavelength			
= 1/5 of tube length	381.5 ± 0.06	381.7 ± 0.18	+0.2
= 1/7 of tube length	535.4 ± 0.06	535.8 ± 0.19	+0.4
= 1/11 of tube length	843.1 ± 0.08	843.1 ± 0.29	0.0
= 1/13 of tube length	998.1 ± 0.05	998.3 ± 0.23	+0.2

The resonant frequencies for 3, 9 and 15 quarter wavelengths in the tube are unaffected, within experimental error, by whether there is a node or an antinode at the bend. The results for 5, 7, 11 and 13 quarter wavelengths in the tube show that when, by reducing its bore, the bend has been caused to behave like the same length of straight tube whether nodal or antinodal, the compensation holds at least roughly for regions that lie between nodes and antinodes.

From these results it seems likely that it would be advantageous in wind instruments to reduce the bore diameter slightly at bends. The amount of reduction required will have to be determined by resonance measurements in each case unless an adequate theory of oscillatory flow round bends can be de-

veloped. The present observations suggest that for the rather tight bends of the butt of a bassoon or the valve slides of a trumpet it will be of the order of 5%.

The "speed of sound" in the ordinary sense is the product of frequency and wavelength, and is the same for travelling waves as for standing waves. If distances are measured along the centre line of the tube, sound goes faster along a toric bend than along a straight tube by a factor which, for the first resonator of Table 1, is about 1.09.

The very accurate toric tubes were made by Mr W. F. Piper.

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BIOLOGICAL SCIENCES

Inhibitor of DNA Polymerase in Sera of Tumour-bearing Mice

WE have previously reported the existence of several DNA polymerases in the murine myeloma line, MOPC-21 (refs 1 and 2). Two of these polymerases, which we refer to as R-1 and R-2, are capable of synthesising poly(dT) on a poly(rA)-oligo(dT)₁₂₋₁₈ template. In this report, we show that the R-1 polymerase shares immunological determinants with the 'reverse transcriptase' of the Rauscher murine leukaemia virus (MLV). In addition, we show that mice inoculated with the MOPC-21 myeloma tumour line or the Rauscher-virus-induced MCDV-12 tumour line contain a non-immunoglobulin factor in their sera which specifically inactivates the R-1 polymerase.

The R-1 and R-2 polymerases were isolated from homogenates of MOPC-21 myeloma tumour by stepwise salt elution chromatography from DEAE-cellulose. Further purification of these fractions was achieved by phosphocellulose chromatography, as previously described^{1,2}. Purified rabbit immunoglobulin directed against the DNA polymerase of the Rauscher murine leukaemia virus was the gift of Drs G. Todaro and E. Scolnick³. Table 1 demonstrates that the R-1 polymerase is inhibited by specific antibody prepared against the MLV polymerase, and that the R-2 polymerase is not affected. This indicates that the R-1 polymerase derived from this tumour line shares common or related immunological determinants with this viral DNA polymerase.

If syngeneic Balb/c mice are inoculated with 10^5 MOPC-21 myeloma cells or comparable numbers of the MCDV-12 tumour line, the serum of these mice contains a factor which inhibits the action of the R-1 polymerase. The time course of appearance of the inhibitory factor is shown in Fig. 1. The sera of syngeneic mice bearing these tumours exhibit a progressive increase in their ability to inhibit the R-1 polymerase. In contrast, the sera of the allogeneic CFW mice receiving tumour cells display only minimal inhibition of the R-1 polymerase, and this inhibition remains essentially level coincident with initial growth of the tumour inoculum and its subsequent rejection. Inoculation of syngeneic spleen cells or repeated administration of the Rauscher MLV does not give rise to the appearance of inhibitor in the sera of Balb/c mice. As Fig. 2a shows, sera from Balb/c mice bearing the MOPC-21 myeloma tumour were as effective as comparable sera from MCDV-

Table 1 Inhibition of the Myeloma R-1 Enzyme by Antibody Specific for MLV Polymerase

	C.p.m. $^3\text{H-TMP}$ incorporated
Experiment 1	
R-1 + IgG (anti-polymerase)	4,469 $\pm$ 174 ($n=2$)
R-1 + IgG (non-immune)	12,740 $\pm$ 636 ($n=2$)
R-1 Control	14,961 $\pm$ 106 ($n=3$)
R-2 + IgG (anti-polymerase)	7,240 $\pm$ 135 ($n=4$)
R-2 + IgG (non-immune)	7,366 $\pm$ 247 ($n=4$)
R-2 Control	8,372 $\pm$ 756 ($n=3$)
Experiment 2	
R-1 + IgG (anti-polymerase)	11,127 $\pm$ 82 ($n=3$)
R-1 + IgG (non-immune)	15,936 $\pm$ 174 ($n=3$)
R-1 Control	16,124 $\pm$ 580 ($n=2$)
RMLV polymerase + IgG (anti-polymerase)	2,111 $\pm$ 56 ($n=2$)
RMLV polymerase + IgG (non-immune)	45,867 $\pm$ 975 ($n=2$)
RMLV Control	44,437 $\pm$ 843 ($n=2$)

Our standard assay for synthesis of poly(dT) by the R-1 polymerase or the Rauscher MLV polymerase using poly(rA)-oligo(dT)₁₂₋₁₈ as template was carried out in a volume of 250 μl . The reaction mixture contained 0.05 M Tris, pH 7.8, 4 mM β -mercaptoethanol (BME), 0.2 mM Mn^{2+} , 200 $\mu\text{g ml}^{-1}$ bovine serum albumin (BSA); $^3\text{H-TTP}$ was supplied at a specific activity of 4,839 c.p.m. pmol^{-1} , and each assay contained 1.08 nmol of TTP and 8.3 nmol of dATP. 0.05 A_{260} units of poly(rA)-oligo(dT)₁₂₋₁₈ was present as template, and the desired amount of enzyme was added to the reaction mixture in 50 μl . Incubation was carried out routinely for 60 min, at which time excess pyrophosphate, 200 μg yeast RNA and trichloroacetic acid were added and the reaction mixture was filtered through a Schleicher and Schuell B-6 membrane filter. The filters were dried and counted in a 0.02 POPOP-0.5% PPO-toluene scintillation mixture. Conditions for assay of the R-2 polymerase were similar except that Mn^{2+} was supplied at 1 mM. All assays were performed in duplicate. In experiment 1, 80 μg of purified rabbit immunoglobulin directed against the MLV polymerase was diluted to 35 μl in enzyme dilution buffer (0.02 M Tris-HCl, pH 7.8; 4 mM BME; 1 mg ml^{-1} BSA and 20% glycerol). A 5 μl sample of this mixture was added separately to 0.83 μg of the myeloma R-1 enzyme and to 0.94 μg of the myeloma R-2 enzyme, each in a total volume of 10 μl , and this mixture was held at room temperature for 30 min. At that time, 40 μl of enzyme dilution buffer was added along with 0.200 ml of assay buffer containing all of the components of our standard assay mixture employing poly(rA)-oligo(dT)₁₂₋₁₈ as template. In experiment 2, 40 μg of anti-polymerase IgG was diluted to 20 μl and 5 μl of this mixture was added separately to 0.83 μg of myeloma R-1 polymerase and to 6.0 μg of the Rauscher MLV polymerase (prepared according to the method of Ross *et al.*⁶) in a total volume of 15 μl . After 30 min at room temperature, 35 μl of dilution buffer was added along with 0.200 ml of assay buffer containing the rest of the reaction components. All incubations were carried out for 60 min at 37° C. As indicated, IgG prepared from a rabbit not exposed to the viral polymerase was run in parallel using 2.4 times the weight of IgG used in the anti-polymerase case. Reaction mixtures containing dilution buffer in place of IgG were also run as controls in parallel. The results are presented as the mean value of the number of observations indicated $\pm$ the standard error of the mean.

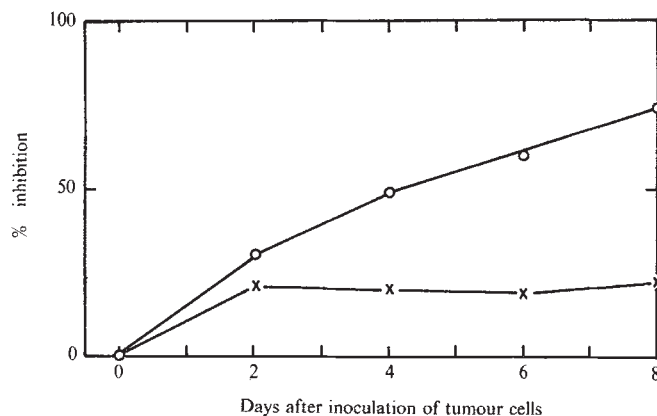


Fig. 1 Time course of development of polymerase inhibitor in serum. Balb/c (○) and CFW (×) mice were inoculated with 10^5 MCDV-12 tumour cells. Aliquots of serum were drawn on the days indicated and 1/8 dilutions prepared in enzyme dilution buffer. A sample of 75 μl of the respective dilutions was added to 8.1 μg of the R-1 polymerase in a total volume of 150 μl . These mixtures were left for 30 min at room temperature at which time 50 μl each of the mixtures was transferred to duplicate assay tubes and incubation continued for another 60 min at 37° C. The specific activity of the $^3\text{H-TTP}$ used in these assay mixtures was 4,570 c.p.m. pmol^{-1} . Under these conditions, this amount of enzyme catalysed the incorporation of 11.4 $\text{pmol } ^3\text{H-TMP h}^{-1}$.

12-bearing hosts in suppressing the activity of the R-1 polymerase. The inhibitor is not present in serum derived from normal mice.

Fractionation of serum from mice bearing the MCDV-12 tumour led to recovery of the inhibitor in the fraction of serum which was soluble in 50% ammonium sulphate solution. This fraction can be shown to be free of detectable immunoglobulin, and this is direct evidence that the inhibitor is not an antibody. Moreover, the inhibitor displays no nuclease activity against either the template or the product of the R-1 polymerase reaction (our unpublished observations). The striking specificity of the inhibitor on the action of the R-1 polymerase is shown in Fig. 2b. The inhibitor had no effect on the ability of polymerases derived from the avian myeloblastosis virus or the Rauscher MLV to synthesise poly(dT) using poly(rA)-oligo(dT)₁₂₋₁₈ as template.

As little inhibitor was found in the serum of mice in which these tumours were able to grow only briefly, we reasoned that the inhibitor was produced by the tumour and was not derived from the host animal. When allogeneic CF1 mice were irradiated so as to depress their immune response to the point where they could no longer reject an inoculum of MCDV-12 tumour cells, substantial amounts of the R-1 polymerase inhibitor were detected in the sera of these irradiated mice after inoculation with the MCDV-12 tumour. This result strongly suggests that the R-1 inhibitor is produced by the tumour cell itself, and that the amount of inhibitor present in the sera of mice bearing the MCDV-12 tumour is proportional to the extent of growth of the tumour in a particular host. This conclusion is supported by the observation that irradiation of the MCDV-12 tumour cells themselves, before inoculation into syngeneic Balb/c mice, prevents the growth of that tumour in these mice, and no inhibition of R-1 polymerase activity is displayed by their sera (Table 2).

It is interesting that although the MCDV-12 tumour was originally induced by the Rauscher MLV, and produces a virus of high virulence, the myeloma line was initially induced with mineral oil and, while the MOPC-21 line contains type C particles resembling the Rauscher MLV, these particles are not particularly efficient at transforming other cells⁴. Nevertheless, both of these tumours produce

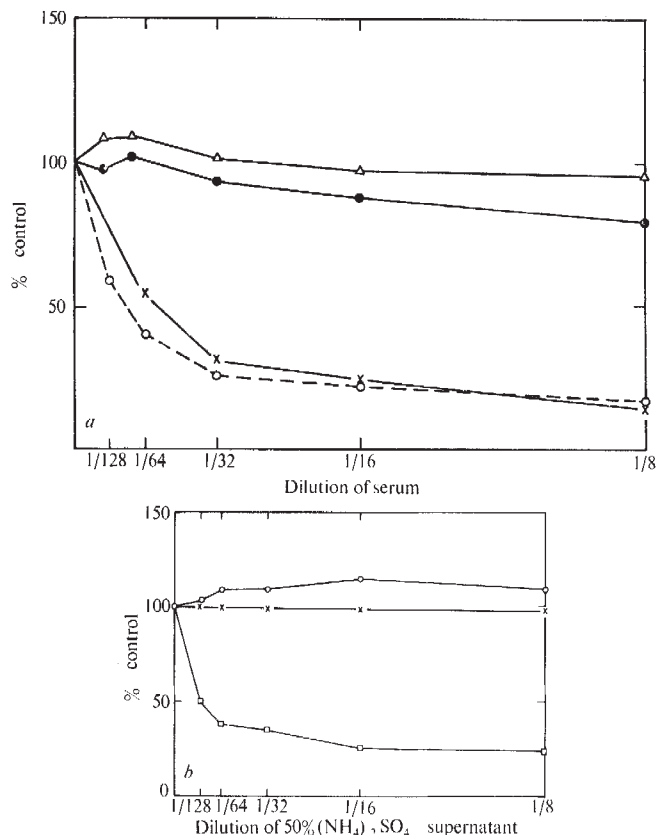


Fig. 2 Specificity of the R-1 inhibitor. *a*, Aliquots of sera from Balb/c mice bearing the MCDV-12 (x) or the MOPC-21 (O) tumour were drawn 7 d after inoculation and diluted as indicated in enzyme dilution buffer. 75 μ l of the respective dilutions of these sera was mixed with 75 μ l of a standard dilution of the R-1 polymerase (165 μ g ml⁻¹), and 50 μ l aliquots of these mixtures were treated as described in Fig. 1. In this study, the amount of enzyme used led to the incorporation of 4.6 pmol of ³H-TMP in the absence of serum. Sera from normal non-tumour-bearing Balb/c mice (Δ) and from CF1 mice that received 10⁵ MCDV-12 cells ($\bullet$) were assayed in parallel. *b*, Sera were collected from Balb/c mice inoculated 7 d previously with 10⁵ MCDV-12 cells. The supernatant fraction soluble in half-saturated (NH₄)₂SO₄ was prepared, and diluted as indicated in enzyme dilution buffer. 75 μ l of these dilutions was added to 75 μ l of enzyme dilution buffer containing 6.2 μ g of the R-1 polymerase ($\square$), or 9.0 μ g of the avian myeloblastosis virus polymerase (x), prepared according to the method of Kacian *et al.*⁷ or the Rauscher MLV polymerase (O). Incubation and assay were carried out as indicated in the legend to Table 1 except that 6 mM Mg²⁺ was substituted for Mn²⁺ in the assays utilising AMV. Under these conditions, in which the specific activity of the ³H-TTP used was 4,570 c.p.m. pmol⁻¹, the amounts of R-1, AMV and MLV polymerases in these assays catalysed the incorporation of 4.78, 7.98 and 4.28 pmol of ³H-TMP respectively. The protein concentration of the half-saturated ammonium sulphate supernatant was 9.8 mg ml⁻¹, as determined by the procedure of Lowry *et al.*⁸.

an inhibitor which is specifically directed against a virus-related polymerase (R-1), and has no effect against other DNA polymerases, including the 'reverse transcriptase' of the Rauscher MLV. In these studies, the appearance of the R-1 inhibitor in serum seems to be correlated with tumour growth. At present, it is not known whether this inhibitor is unique to tumour cells or might, for example, be produced by normal cells which are growing rapidly. Moreover, the fact that such an inhibitor inactivates a cytoplasmic polymerase which is immunologically related to the DNA polymerase from Rauscher MLV, but has no effect on the viral polymerase itself, points out the necessity for caution in studies which attempt to distinguish between cellular and viral polymerases present in tumour cells.

It is particularly important to reinforce the distinction between immunoglobulin directed against a viral DNA

Table 2 Effect of Irradiation of Cells and Recipient Mice on Appearance of R-1 Polymerase Inhibitor in Serum

Serum from mice	C.p.m. ³ H-TMP incorporated	% Control
Experiment 1 (CF1 mice)		
Irradiated and given MCDV-12 cells	9,128	19.8
MCDV-12 cells alone, no radiation	33,986	73.8
Irradiated and given SRBC	44,540	96.8
SRBC alone, no radiation	44,856	97.5
Prior to being irradiated	49,162	106.8
Enzyme control (no serum)	46,027	100.0
Experiment 2 (Balb/c mice)		
Given irradiated MCDV-12 tumour cells	32,038	81.7
Given unirradiated MCDV-12 tumour cells	16,155	41.2
Normal, no treatment	34,730	80.9
Enzyme control (no serum)	39,212	100.0

In experiment 1, CF1 mice were irradiated with 700 r. before receiving 10⁵ MCDV-12 tumour cells of 1 $\times$ 10⁸ sheep red blood cells (SRBC). An equal number of mice was not irradiated and was challenged with MCDV-12 tumour cells and (or) SRBC. Serum was collected 7 d after challenge and pooled separately from both sets of mice and 75 μ l aliquots of a (1/8) dilution of these sera were mixed with 10.7 μ g of the R-1 polymerase in a total volume of 150 μ l, and held at room temperature for 30 min. Aliquots of 50 μ l of these mixtures were assayed in our standard assay system. Samples of sera drawn before irradiation were tested in parallel. In experiment 2, six Balb/c mice were inoculated with 7.5 $\times$ 10⁷ MCDV-12 cells which had been previously irradiated with 1,000 r. A separate set of six mice was given 10⁵ unirradiated MCDV-12 cells. Sera were obtained after 7 d, diluted 1/8 with enzyme dilution buffer, and mixed with 10.7 μ g of R-1 polymerase for 30 min at room temperature. Aliquots of 50 μ l were then removed and tested for residual polymerase activity. Sera from normal Balb/c mice were pooled and assayed in parallel.

polymerase and non-immunoglobulin inhibitory factors such as the one described herein which can selectively inactivate specific DNA polymerases.

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Use of ^{125}I in Fingerprinting RNA

It is often difficult to obtain highly radioactive *in vivo* ^{32}P -labelled RNAs from higher organisms for fingerprinting¹. In such situations, *in vitro* labelling methods can make a valuable contribution. One method using polynucleotide phosphokinase to introduce ^{32}P at the 5' terminus of oligonucleotides has been reported^{2,3}. Here I describe another method which is based on the fact that iodine has a high

slower in the second relative to unmodified oligonucleotides (Fig. 1a), indicating a greater net negative charge on them in agreement with the observed mobility of iodinated cytidylic acid⁷.

The results demonstrate the usefulness of ^{125}I for fingerprinting RNAs. The method is especially attractive because of its specificity, simplicity, speed and sensitivity. The fact that a fingerprint of a 'cold' RNA can be easily obtained by this method makes it a very useful procedure for studying

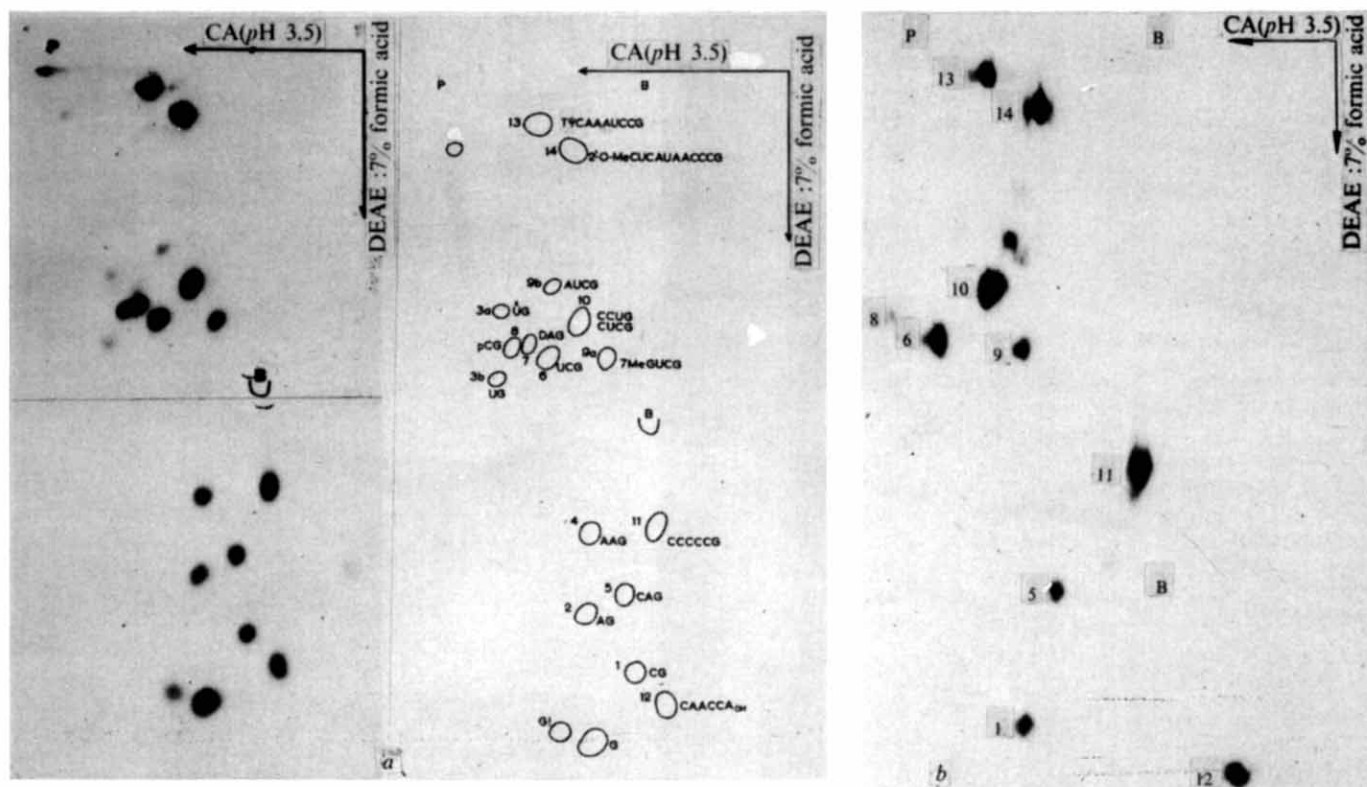


Fig. 1 A two-dimensional fractionation of an RNase T_1 digest of *in vivo* ^{32}P -labelled, (a), and *in vitro* ^{125}I -labelled, (b), *E. coli* tRNA^{Met}. tRNA^{Met} was prepared as described⁸. The tRNA was labelled with ^{125}I in a total volume of 2 μl containing 0.2 μg tRNA, 10 mM sodium acetate acetic acid buffer, pH 5.0, 0.01 mM KI equilibrated with 10 to 100 μCi Na^{125}I (Amersham, England) and 0.1 mM TiCl_3 (Merck, Germany). The reaction mixture assembled at 0° C, with reagents added in the above order, was taken up in a siliconised capillary tube which was afterwards sealed at both ends and incubated at 80° C for 10 min. The capillary tube was cooled on ice and broken open. Sodium thiosulphate (final concentration 1 mM) was added to reduce excess iodine and TiCl_3 . Then 20 μg carrier RNA (*E. coli* mixed tRNA) and ammonium acetate ammonia buffer, pH 9 (final concentration 0.2 M), was added. The capillary tube was sealed again at both ends and heated at 45° C for 2 h to convert the unstable 5-iodo-6-hydroxy-5,6-dihydropyrimidines back to the unsaturated form. After this the RNA was passed over a Sephadex G-25 column made out of a siliconised capillary tube. The fraction eluting at the void volume was precipitated with ethanol. The tRNA was digested with RNase T_1 with an enzyme to substrate ratio of 1 : 20 for 30 min at 37° C¹. The digest was fractionated on a two-dimensional high voltage electrophoretic system¹ using cellulose acetate, pH 3.5, for the first dimension and DEAE:7% formic acid for the second. The electrophoretogram was autoradiographed using Kodak X-Omatic H Film. The autoradiograph of the ^{125}I -labelled tRNA was obtained by exposure of the film for 8 to 12 h. The diagram shows the positions of the oligonucleotides (a) with their identification numbers and sequences⁸. B is the blue marker; P is the dye marker peak.

specificity for cytosine under certain conditions⁴ and that iodine isotopes (^{125}I , ^{131}I) are easily detectable by autoradiography. I have used *Escherichia coli* formyl-methionine tRNA of known sequence^{5,6} to test the suitability of this method for fingerprinting RNAs. The results show that a fingerprint of pmol amounts of RNA is obtainable within 2 d.

Figure 1b shows an RNase T_1 fingerprint of iodinated tRNA^{Met} and Fig. 1a that of the *in vivo* ^{32}P -labelled tRNA^{Met}. The accompanying diagram gives the nucleotide sequences of the oligonucleotides. Several points in the figure are noteworthy. The fingerprint of iodinated tRNA is identifiable with that of the ^{32}P -labelled tRNA. Only cytosine containing oligonucleotides are prominent on the fingerprint and their intensity is in good agreement with their cytosine content indicating that, in the conditions used for labelling, the secondary structure of RNA is sufficiently opened up to make possible uniform iodination. The iodinated oligonucleotides move slightly faster in the first dimension and

homologies between closely related RNAs from different species, cell types and RNA tumour viruses.

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Repair Endonuclease-sensitive Sites in Daughter DNA of Ultraviolet-irradiated Human Cells

WE have used cells that were derived from individuals with xeroderma pigmentosum (XP) and are defective in excision repair¹ to demonstrate the appearance of ultraviolet lesions in the DNA made after ultraviolet irradiation. Normal human and XP cells were irradiated and labelled with either ³H-thymidine or ³²P₄ for 6–8 h, the label was removed, and the cells were further incubated for 18 h to allow for DNA chain elongation and joining of the short DNA segments synthesised after irradiation. The cells were then osmotically shocked, treated with a repair endonuclease contained in *Micrococcus luteus* extract for 5 min at 37° C and pH 8.0, and sedimented in 5–20% alkaline sucrose gradients. The enzymatic assay² and the sedimentation procedure³ have been described elsewhere. Figure 1 shows that daughter DNA from irradiated and non-irradiated normal human cells contains no repair endonuclease-sensitive sites. This is expected because normal human cells excise and repair most of the pyrimidine dimers formed after ultraviolet irradiation⁴. Figure 2 shows that DNA synthesised by irradiated XP cells does contain repair endonuclease-sensitive sites, although the number appears to be small. Under our experimental conditions there is approximately one sensitive site for every fifteen to thirty-five dimers in the parental DNA⁵.

The repair endonuclease-sensitive sites could result from (a) DNA exchanges between parental and daughter strands, (b) insertion of abnormal bases into the DNA made from irradiated templates and the recognition of these abnormal bases by the repair endonuclease, or (c) recognition of the dimer but incision into the DNA strand not containing the dimer.

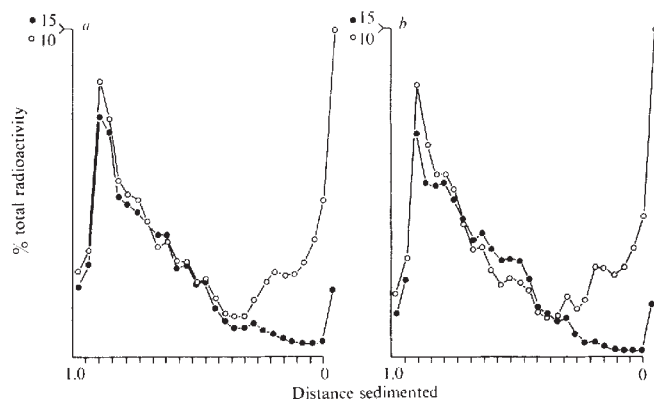


Fig. 1 Alkaline sucrose gradient profiles of DNA from cells of normal human cell line HSWP which were ultraviolet-irradiated with 150 erg mm⁻² (●) or non-irradiated (○) incubated in 12.5 μCi ml⁻¹ of ³H-thymidine (specific activity 18.1 Ci mmol⁻¹) or 30 μCi ml⁻¹ of ³²P₄, respectively, for 8 h. The label was removed and the cells were incubated for 18 h before (a) sedimentation or (b) treatment with repair endonuclease extract from *M. luteus* and sedimentation. Sedimentation was for 180 min at 25,000 r.p.m. in a 2 M salt–5–20% alkaline sucrose gradient. The ³H and ³²P profiles in both (a) and (b) were co-sedimented. Total c.p.m. (a, ●) 3,897; (a, ○) 14,832; (b, ●) 2,612; (b, ○) 11,631.

DNA exchanges between parental and daughter strands have been observed in irradiated *Escherichia coli* cells analysed in CsCl density gradients⁶, and repair endonuclease-sensitive sites have also been observed in the daughter DNA of irradiated *E. coli* cells (A. Ganesan, unpublished observations). However, attempts to demonstrate DNA exchanges between parental and daughter strands in mitotic cells (ref. 7 and S. N. B. and J. D. R., unpublished observations) or somatic recombination (ref. 8 and G. Pontecorvo, personal communication) in mammalian cells have been unsuccessful.

Previously we showed that in both normal human and XP

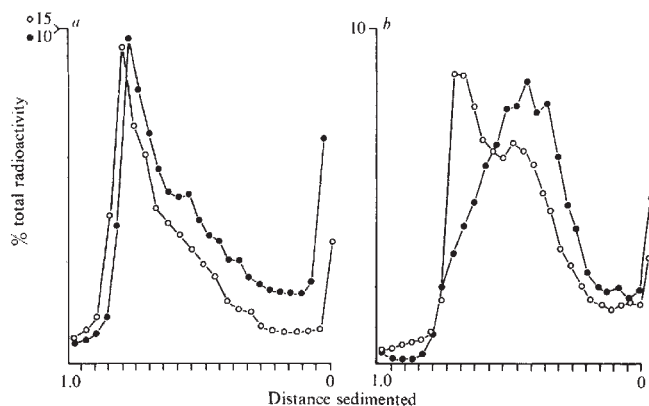


Fig. 2 Alkaline sucrose gradient profiles of DNA from cells of XP cell line SG-XP4 which were ultraviolet-irradiated with 150 erg mm⁻² (●) or non-irradiated (○) and incubated in 25 μCi ml⁻¹ of ³H-thymidine (specific activity 18.1 Ci mmol⁻¹) for 8 h. The label was removed and the cells were incubated for 18 h before (a) sedimentation or (b) treatment with repair endonuclease from *M. luteus* extract and sedimentation. Sedimentation was for 150 min at 25,000 r.p.m. in a 2 M salt–5–20% alkaline sucrose gradient. Each profile was individually sedimented. Total c.p.m. (a, ●) 6,489; (a, ○) 8,312; (b, ●) 5,440; (b, ○) 3,300.

cells DNA synthesised from ultraviolet-irradiated templates contains discontinuities⁹, and that ~75% of the discontinuities are completed by insertion of exogenous nucleotides¹⁰. During completion of the discontinuities, abnormal bases could be inserted, and the resulting structural abnormalities could be recognised by endonucleases in the *M. luteus* extract, so that incisions would be made in the DNA. The insertion of such abnormal bases could lead to accumulation of errors in the DNA and result in the XP phenotype, which is characterised by the appearance of skin cancers at a very early age¹¹. Although previous results show that the repair endonuclease is specific for the strand containing the dimer^{12,13}, minor non-specificity results in one incision in the strand not containing the dimer for every seventy dimers in the complementary strand¹³.

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Differences in Murine Leukaemia Virus-specific DNA Sequences in Normal and Malignant Cells

In many mouse strains, RNA tumour virus information is transmitted vertically (inherited) perhaps as part of the cell genome^{1,2}. The activation of type C virus replication in cells from low leukaemia incidence mouse strains³ and from 'virus-free' cell lines⁴ by halogenated pyrimidines suggests that all mouse cells may contain murine leukaemia virus (MuLV) information, often in an unexpressed form. Indeed, proponents of the 'oncogene' hypothesis have predicted a complete copy of type C virus information in the genome of all somatic cells⁵.

Varmus *et al.*⁵, using acceleration of reannealing kinetics of radioisotopically labelled double stranded DNA product from a reverse transcriptase reaction, by unlabelled cell DNA, have shown that the amount of virus information was the same in the DNA of cells from low and high mammary tumour (MMT) incidence strains. Using similar techniques, Gelb *et al.*⁶ failed to demonstrate a difference in viral DNA content of normal and virus-transformed cells. Similar results have been obtained using an avian oncornavirus system⁷. Thus, if RNA tumour virus information is similar in mouse strains with different tumour frequencies and in normal and transformed cells, oncogenesis would depend on factors in addition to virus structural genes (for example, regulatory genes, biological or chemical agents or ageing).

In an attempt to further characterise leukaemia virus DNA content in mouse cells we have synthesised *in vitro* a radioactively labelled single stranded DNA copy of the naturally occurring AKR (Gross) murine leukaemia virus (AKR-V) and the Rauscher leukaemia virus (RLV). These probes were used in DNA-DNA hybridisation and saturation experiments with DNA from normal cells of low and high leukaemia incidence mouse strains and virus-induced lymphomas. Our results indicate quantitative and qualitative differences among the various cellular DNA preparations as demonstrated by extent of hybridisation, reannealing kinetics and thermal stability of the various DNA duplex hybridisation products.

Viral ³H-DNA was synthesised by an endogenous reverse transcriptase reaction in the presence of actinomycin D, in order to inhibit viral and host DNA-directed DNA synthesis. The viral probe was purified by utilising phenol-cresol, alkali hydrolysis of RNA, and Sephadex and hydroxyapatite chromatography⁸. The probes were self-annealed⁹ and less than 0.5% was found to be double stranded. Both viral ³H-DNA probes had a sedimentation coefficient of 5–8S in an alkaline sucrose equilibrium gradient. They hybridised completely to purified viral RNA and not to synthetic polyribonucleic acid. The latter hybridisation was done specifically to rule out the possibility of transcription from polyadenylic acid regions of the viral genome¹⁰. Cell DNA was extracted from a high incidence mouse strain (AKR) and low incidence strains (Balb/c, NIH Swiss) and Rauscher and Gross virus-induced lymphomas by the method of Britten *et al.*¹¹ using batch elution from hydroxyapatite powder followed by mechanical shearing to a piece size comparable with the viral ³H-DNA. All cell DNA reannealed completely after denaturation. Hybridisation experiments were carried out to a C_0t value of 3,000, which represented the terminal plateau of the reannealing curve (see below). C_0t equals starting DNA concentration $\times$ time (mole $\times$ s l⁻¹), as defined by Britten and Kohne⁹.

Table 1 shows more extensive hybridisation of AKR-³H-DNA than RLV-³H-DNA with all of the mouse DNA preparations tested. The percentage of the AKR probe hybridised was greatest with lymphoma DNA and succes-

sively less than normal AKR, Balb/c and NIH Swiss DNA. In hybridisation experiments using RLV-³H-DNA, we were unable to demonstrate differences in mouse strains. There was, however, more extensive hybridisation of the RLV probe to lymphoma than to normal DNA. Reannealing curves, which also reflect the concentration of complementary sequences in the cell DNA, demonstrated more rapid reannealing of the AKR probe with lymphoma and normal AKR DNA than with NIH Swiss DNA (Fig. 1). A plateau is reached at a C_0t value of approximately 3,000, after which no further hybridisation of the AKR-³H-DNA with any of the cell DNA preparations could be detected.

Table 1 Hybridisation of Virus-directed ³H-DNA with Cellular DNA

	AKR- ³ H-DNA			RLV- ³ H-DNA		
	% Hybridised 60° C	80° C	$T_m(e)$ (°C)	% Hybridised 60° C	80° C	$T_m(e)$ (°C)
Normal mouse DNA						
AKR	50.1	23.5	79.5	22.2	7.5	75.5
Balb/c	40.0	13.6	77.5	22.5	5.1	73.0
NIH Swiss	35.5	8.3	75.0	20.1	7.6	74.0
Lymphoma DNA						
Gross	54.6	27.7	80.5	35.6	12.6	78.0
Rauscher	59.9	25.8	78.5	48.9	20.2	77.5
Salmon sperm DNA	1.0	0	< 65	2.4	0	< 65

Hybridisation of viral ³H-DNA probes with cell DNA. RLV was obtained from the plasma of infected mice²¹; AKR-V was obtained from Electronucleonics Corp. Virus (250 µg protein) in 150 µl Tris (hydroxymethyl aminomethane), pH 8.3, was ruptured by incubating for 10 min at 4° C in the presence of 0.003% Nonidet P-40 (Shell) and 30 mmol dithiothreitol. The virus sample was made to a volume of 375 µl with an incubation mixture containing: 19.2 mmol Tris, pH 8.3, 18 mmol KCl, 3.1 mmol MgCl₂, 0.2 µmol dATP, dCTP, and dGTP, and 0.6 mCi ³H-TTP (specific activity 24,000 c.p.m. pmol⁻¹), and 37 µg actinomycin D, and incubated for 45 min at 37° C. The reaction was terminated by addition of NaCl and sodium dodecyl sulphate (SDS) to a final concentration of 0.4 M and 1%, respectively. An equal volume of phenol-cresol (7:1) containing 0.4 g 8-OH quinoline per 100 ml was added to the sample, mixed and centrifuged at 5,000g for 5 min at 25° C. The aqueous phase was removed and placed on a Sephadex column (G50, medium) with a total bed volume of 100 ml; 0.5 ml fractions were collected and fractions from the DNA region of the gradient were pooled. NaCl (to 0.4 M) and 10 µg ml⁻¹ carrier yeast RNA were added and the nucleic acid precipitated with 2 volumes of cold ethanol for 12 h at -20° C. The solution was centrifuged at 12,000 r.p.m. for 30 min at 4° C and the pellet resuspended in 100 µl 0.3 M NaOH and kept at 37° C for 18 h to hydrolyse RNA. The sample was neutralised with HCl and brought to 0.024 M in phosphate buffer (PB) containing equimolar parts of Na₂HPO₄ and NaH₂PO₄, pH 6.8. The sample was passed through a hydroxyapatite column (2 cm deep and 1 cm in diameter) at 60° C, and eluted with 0.4 M PB. The nucleic acid preparation obtained was self-annealed for 96 h at 60° C and double stranded DNA separated using a hydroxyapatite column²². Normal adult livers were used as a source of AKR, Balb/c and NIH Swiss DNA. Lymphomatous DNA was obtained from virus-induced tumours which were serially transplanted in low-leukaemia incidence strains (Gross lymphoma in C57/6 bl, Rauscher lymphoma in Balb/c). Salmon sperm DNA was purchased from Sigma. Whole cell DNA was extracted by the method of Britten *et al.*¹¹ which consists of homogenising tissue in the presence of urea, PB and sodium perchlorate, extraction with chloroform-octanol (24:1) and purification by hydroxyapatite chromatography. DNA fragments were prepared by mechanical shear (50,000 pounds per square inch) and passed through cellulose acetate filters. The ratios of A_{260} to A_{280} of the DNA preparations were all close to 2. Annealing reaction mixtures contained 6 A_{260} units of cell DNA, 0.5 pmol of ³H-DNA, 2% SDS, 10⁻³ EDTA in 500 µl PB adjusted to 0.5 M. The mixture was heated for 10 min at 100° C and placed in a 60° C water bath for 120 h ($C_0t = 3 \times 10^3$)⁹. The sample was then diluted to 0.14 M PB and passed over a hydroxyapatite column (2 cm in depth) previously equilibrated with 0.14 M PB containing 4% SDS at 60° C. The double stranded DNA was eluted with 0.4 M PB and radioactivity counted in a liquid scintillation counter using Instagel (Packard) scintillation fluid. Thermal elutions were performed by loading the column with annealing mixtures diluted to 0.14 M PB and raising the temperature of the column by 5° C increments from 60° to 100° C and eluting with 2 ml 0.14 M PB and counting radioactivity as described above. The percentage hybridisation at 80° C refers to the fraction of the probe remaining double stranded (hybridised) when the column temperature was raised to 80° C.

The differences in extent of hybridisation and reannealing kinetics among the DNA preparations tested indicate quantitative differences in MuLV information in cell DNA. The thermal stability profiles (Fig. 2) of the hybrid DNA duplexes indicated that there are qualitative differences also. $T_{m(e)}$, derived from the thermal elution curves, represents the temperature at which 50% of the double stranded DNA is eluted from the hydroxyapatite column and correlates well with T_m obtained by optical methods¹². The $T_{m(e)}$ of hybrids formed with the AKR probe was higher than those formed with the RLV probe for all DNA samples tested. Haapala and Fischinger¹⁸ have reported considerable molecular divergence among the genomes of various mouse leukaemia viruses. The higher $T_{m(e)}$ obtained with AKR-³H-DNA hybrids possibly represents more precise base pair matching with the naturally occurring MuLV probe.

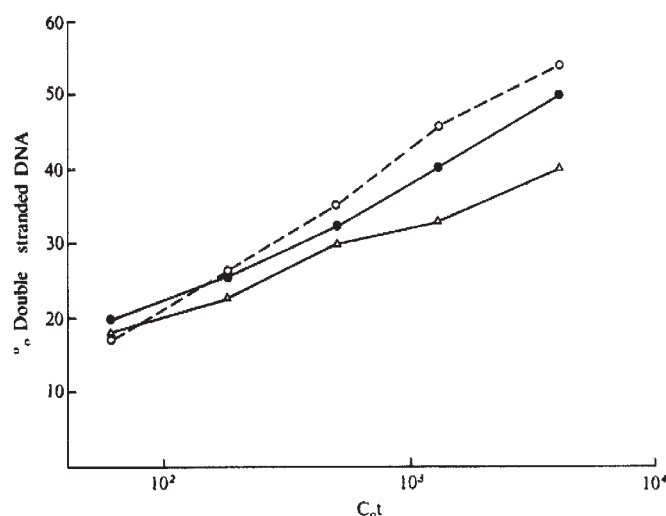


Fig. 1 Annealing curves of AKR-³H-DNA with Gross lymphoma (O), AKR (●), and Balb/c (▲) DNA. Each point represents a 100 μ l aliquot removed from a 0.5 ml reaction mixture incubating at 60° C. Double stranded DNA was determined by fractionation on hydroxyapatite as described previously.

The hybrids formed with AKR-³H-DNA, in order of decreasing $T_{m(e)}$, were Gross lymphoma > AKR > Rauscher lymphoma > Balb/c > NIH Swiss DNA. The thermal stability of polynucleotide duplexes depends on their length, base composition and relative composition of complementary base pairs. Since the piece sizes of cell DNAs were similar, the higher thermal stability of duplexes formed by AKR-³H-DNA with lymphoma and AKR DNA compared with Balb/c and NIH Swiss DNA indicates more precise base pairing and (or) the existence of additional base pairs with a high G:C content in the former DNA preparations.

Thus, one possibility is that there was a greater degree of molecular divergence (that is, mutation) of the MuLV nucleotide sequences in the low incidence mouse strain genome accounting for the lower $T_{m(e)}$ of the hybrid formed with the AKR probe.

Another possibility is that AKR and lymphoma DNA contain additional information not present in low leukaemia incidence mouse DNA; recycling experiments¹⁴ were performed to test this possibility. AKR-³H-DNA was extensively hybridised to NIH Swiss DNA. NIH Swiss DNA was present in great excess and the reaction was taken to a C_0t value of 10,000 at 60° C. This is a C_0t value where all complementary probe sequences are saturated with cell DNA, and no additional hybridisation of the probe

will occur when incubated with NIH Swiss DNA. The non-hybridised fraction of the probe was separated using a hydroxyapatite column and incubated with AKR and Gross lymphoma DNA and 2.5% and 6.4% of the recycled probe hybridised, respectively. Thus, it seems that AKR probe sequences are present in normal AKR and lymphoma DNA that are not present in NIH Swiss DNA.

A critical factor in experiments using ³H-DNA probes synthesised *in vitro* is the possibility of asymmetrical transcription with certain viral sequences being transcribed preferentially and others not at all⁶. This might account for the differences we observed between the two viral probes. Nevertheless, using probes synthesised in this manner, the AKR probe may offer certain advantages over the RLV probe. RLV-³H-DNA has previously been used extensively in detecting complementary MuLV sequences in other species^{8,15,16}.

These results contrast with previous reports demonstrating little difference in tumour virus DNA content in normal and neoplastic cells^{6,7} and in low and high tumour incidence strains^{5,17}. Gelb *et al.*¹⁷ were unable to find a difference in the number of MuLV genome equivalents in mouse embryo DNA from different strains using acceleration of reannealing kinetics of Kirsten leukaemia virus-specific double stranded DNA. In our experiments adult liver was used as a source of cellular DNA. AKR embryos are free of detectable MuLV; virus replication begins shortly after birth in certain cells, viraemia occurs and an exogenous viral infection probably occurs in liver cells¹⁸. This phenomenon could explain the quantitative differences we observed in MuLV-specific DNA in AKR and NIH Swiss DNA, but does not adequately explain the differences in $T_{m(e)}$ of hybridisation products and the results of recycling experiments we observed between these two strains.

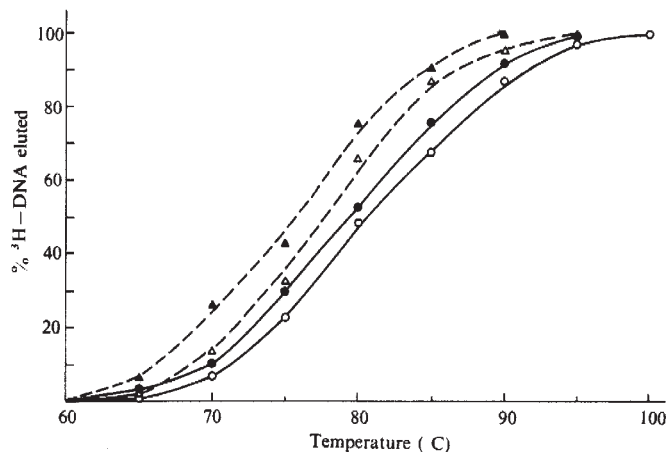


Fig. 2 Thermal stability curves of DNA duplex formed by AKR-³H-DNA probe and cell DNA hybridisation. The methods are described in the legend to Table 1. Cell DNAs are normal AKR (●), Balb/c (▲), NIH Swiss (▲) and Gross lymphoma (O).

Balb/c and NIH Swiss cells contain sufficient information for MuLV replication^{3,3}. It has not been determined if the differences between AKR and the two low leukaemia incidence strains we have detected in these hybridisation experiments represent additional information essential for neoplastic transformation, which low incidence strains are lacking. There is no evidence so far that chemically induced type C viruses from low tumour incidence strains of any species are leukaemogenic^{19,20}. Therefore, it is possible that some mouse cells contain information for infectious type C viruses that are non-oncogenic.

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greatly increased metastatic potential. These cells also grow in culture and retain their respective metastatic or non-metastatic character^{1,2}. These cell lines are particularly useful for studying metastasis since all cells are tumorigenic, and one is therefore not faced with making a choice of an appropriate 'normal' cell or tissue to act as a control with which to compare neoplastic or metastatic potential. Dr Fidler kindly supplied us with cell lines 26 (low metastasis) and 37 (high metastasis). Culture conditions and maintenance were as described¹. Since metastasis would seem intuitively to be intimately concerned with plasma membrane properties, we initiated extensive studies on cell surface properties of these cells. We report here the results of these studies and some findings relating to cell growth that may define the process of cell metastasis. It should be pointed out, however, that under the conditions defining the cell lines, implantation, more than metastasis, is being studied. Metastasis implies the ability of a cell to leave a given tumour site, be transported to another site, and implant and grow there; in the studies described here only the last two conditions of metastasis obtain and hence implantation properties are probably being studied.

First we injected 67,000 line 26 cells (in 0.2 ml of saline) into the tail vein of each of five C57 mice and 45,000 line 37 cells (in saline) into five C57 mice. The cells were over 95% viable and were counted on a Coulter Counter Research Model B. Controls received 0.2 ml of saline. Three weeks later, at killing, controls had 0, line 26 had 1.8 ± 0.1 , and line 37 had 11.4 ± 0.5 melanin-containing tumour foci in the lungs (significant at $P < 0.01$). Thus line 37 had produced more than five times as many tumour foci as had line 26, even though 50% more line 26 cells had been inoculated into each mouse.

At first we confined our work to cells that were just confluent (40×10^3 cells cm^{-2}) but found that in many of the experimental parameters related to cell surfaces there was little or no difference between line 26 and line 37 (see below). We therefore looked at sparse cultures so as to better characterise the system. Cell line 26 had a doubling time of 20 h and cell line 37 had a doubling time of 18 h; cell volumes were essentially the same as recorded by the Coulter Counter Volume Plotter. 'Confluent cells' are defined here as cells harvested when they begin to touch each other on the growth plate, a density of about 40×10^3 cells cm^{-2} . 'Sparse cells' are cells collected at a density of 0.8×10^3 cells cm^{-2} . Intermediate cell densities were also studied, and results were found to be essentially intermediate between results for sparse and confluent cultures and were related to cell culture density. Protein determinations³ and cell counts showed that, whether cultures were sparse or confluent, cell line 26 had $1.6 \pm 0.1 \times 10^{-6}$ mg of protein per cell, whereas line 37 had $1.2 \pm 0.1 \times 10^6$ mg of protein per cell.

It soon became evident that the surface properties of the two cell lines were very different. This difference could be due to either one of two parameters: the absolute age of the cell when collected, or the age or state of the interaction

Biochemical Parameters correlated with Tumour Cell Implantation

FIDLER¹ has described melanoma cell lines produced by serial injection into and collection from C57 mice, some lines of which produce few pulmonary metastases while others have

Table 1 Electrophoretic Mobilities of Cells

Condition	Electrophoretic mobility ($\mu\text{m}^{-1} \text{V}^{-1} \text{s}^{-1} \text{cm}$)			
	26 Sparse	37 Sparse	26 Confluent	37 Confluent
Control	-2.10 ± 0.04	-2.77 ± 0.06	-2.11 ± 0.03	-2.07 ± 0.04
Neuraminidase *	-1.95 ± 0.02	-2.11 ± 0.04	-1.92 ± 0.04	-1.90 ± 0.06
20 μg	-1.54 ± 0.04	-1.69 ± 0.11	-1.46 ± 0.07	-1.40 ± 0.10
50 μg	-1.09 ± 0.02	-1.10 ± 0.04	-1.08 ± 0.09	-1.05 ± 0.08
50 μg , boiled	-2.11 ± 0.05	-2.78 ± 0.07	-2.10 ± 0.04	-2.10 ± 0.06
Trypsin *, 50 μg	-2.19 ± 0.07	-2.89 ± 0.04	-2.26 ± 0.06	-2.20 ± 0.07
Hyaluronidase *, 50 μg	-2.04 ± 0.03	-2.64 ± 0.02	-2.01 ± 0.04	-2.00 ± 0.10

Values are means $\pm$ s.e.m. and are for cells measured in 0.0145 M NaCl, 4.5% sorbitol, 0.6 mM NaHCO_3 , pH 7.2 ± 0.1 at $25^\circ \text{C} \pm 0.1$, as previously described^{5,15}.

* Enzyme treatment given as amount per mg cell protein.

Table 2 Partitioning of Cells in Two-phase Aqueous Polymer System

Density	Partition ratios*	
	Cell line 26	Cell line 37
Sparse	0.361	0.432
Confluent	0.174	0.180

Cells were partitioned in a two-phase aqueous polymer system as described by Walter *et al.*^{7,8}. The system was composed of 5% (w/w) Dextran T500 (Pharmacia; Lot No. 1342), 3.5% (w/w) polyethylene glycol (Carbowax 6,000, Union Carbide), equimolar amounts of NaH_2PO_4 and Na_2HPO_4 (total concentration 0.10 M, pH 6.98), and 0.05 M NaCl. Cells were partitioned in 15 × 150 mm test tubes for 1 h at 5° C. Cell number was determined in aliquots of total system before partition and top phase after partition, using a Coulter Counter Model ZB with a 100 μm aperture and Isoton (Coulter) as electrolyte. Partition ratios were calculated as top phase cell number divided by total system cell number.

* Means of four observations each, consisting of ten independent partitions.

between the cell and its support (glass) at the time of collection. Our data suggest that the major surface property differences occur in sparse cultures and that implantation potential is manifest primarily during this time period, inasmuch as surface properties of confluent cells of the two lines seem to be similar.

Electrophoretic mobilities of the two cell lines under various conditions are shown in Table 1, as follows: (1) Sparse cells of line 37 had a much higher electrophoretic mobility than sparse cells of line 26, but confluent cells of both lines had essentially the same mobility. (2) Treatment of all cells with neuraminidase reduced their electrophoretic mobility, and when sparse 37 cells were treated with 50 μg of neuraminidase per mg of cell protein, their mobility was equivalent to that of 26 cells so treated. Measurement of released sialic acid⁴ showed that treatment with 50 μg of neuraminidase per mg of cell protein released 2.5 ± 0.1 μg of sialic acid per mg of cell protein from sparse 37 cells but only 1.0 ± 0.1 μg from sparse 26 cells or confluent 37 and confluent 26 cells. (3) Trypsin treatment and hyaluronidase treatment had little effect on cell mobilities in any of the lines tested; interpretation of similar data for other cell lines is given elsewhere⁵. Thus, when sparse, line 37 had a much higher electrophoretic mobility than sparse 26 cells, and 2.5 times as much neuraminidase-susceptible surface sialic acid.

A more sensitive parameter than whole-cell microelectrophoresis is partition of cells into two-polymer aqueous phase systems, whereby not only surface charge but also some other, poorly defined phenomena are measured⁶⁻⁸. Using

this technique, we found that in sparse culture, line 37 had a much higher partition ratio than line 26 (Table 2), probably indicating, in part, a higher charge⁷. Confluent cells of the two lines had similar partition ratios, but these values were vastly different from the values for sparse cells of either line. Thus, as measured by this parameter, both cell lines show marked differences in cell surface properties between the sparse and confluent cell cultures.

One biochemical change considered in implantation or metastasis is increased levels of degradative enzymes, presumably to enhance the infiltrative capacity of the cell and perhaps for the phenomenon of sublethal autolysis^{9,10}. The data in Table 3 for sparse cultures indicate that line 37 had generally elevated levels of glycosidases and proteases (α -mannosidase and acid phosphatase levels were similar to those of line 26). Confluent cells of lines 26 and 37 had levels of activity similar to those of sparse line 26 (not shown in the table).

Table 4 compares the activity levels of glycoprotein: glycosyl transferases in cells of both lines, using endogenous or exogenous acceptors and based on a whole cell Triton X-100 homogenate. In the line 37 cells the activity levels of many of these transferases were elevated above the levels in the 26 cells. The highest elevations were in the sparse cultures, but elevations were clearly evident in the confluent 37 cells compared to the confluent 26 cells.

Table 4 Total Glycoprotein: Glycosyl Transferase Activities of Cell Lines

Transferase	Cell line			
	Sparse 26	Sparse 37	Confluent 26	Confluent 37
	(c.p.m. per 10 ⁶ cells)			
Collagen: glycosyl*	7,870	27,750	10,460	20,700
Endogenous glucose	8,200	17,960	8,200	8,600
Fetuin: sialyl†	11,320	35,760	10,690	20,490
Endogenous sialyl	7,460	14,100	5,530	15,400
Fetuin: galactosyl‡	17,570	69,000	19,100	42,200
Endogenous galactosyl	40,400	49,200	41,660	31,100
Fetuin: N-acetylglucosaminyl§	9,470	28,170	8,860	24,980
Endogenous N-acetylglucosaminyl	10,320	22,460	13,600	21,890

Enzyme assays were carried out on 0.1% Triton X-100 extracts of the sparse or confluent cells. Details of enzyme assay, preparation of acceptor and complete systems for assay are as given previously^{18,21}. Endogenous activities are without added acceptor in the reaction. Data are means from five experiments.

* Acceptor: Collagen minus glucose¹⁸.

† Acceptor: Fetuin minus N-acetylneuraminic acid¹⁹.

‡ Acceptor: Fetuin minus N-acetylneuraminic acid, galactose²⁰.

§ Acceptor: Fetuin minus N-acetylneuraminic acid, galactose, N-acetylglucosamine²¹.

Table 3 Proteolytic, Glycosidase, and Acid Phosphatase Activities of Cells

Enzyme	Cell line 26 (nmol per h per 10 ⁶ cells)	Cell line 37 (nmol per h per 10 ⁶ cells)
β -D-galactosidase (EC 3.2.1.23)	14 ± 2	68 ± 14
β -L-fucosidase (EC 3.2.1.38)	119 ± 27	339 ± 49
N-acetyl- β -D-galactosaminidase (EC 3.2.1.—)	877 ± 121	2,388 ± 240
N-acetyl- β -D-glucosaminidase (EC 3.2.1.30)	626 ± 78	1,511 ± 129
α -D-mannosidase (EC 3.2.1.24)	175 ± 29	194 ± 48
Acid phosphatase (EC 3.2.3.2)	606 ± 121	610 ± 68
Proteolytic pH 3.4 (cathepsin-like)	28 ± 3.6	69 ± 8.9
Proteolytic pH 7.4 (trypsin-like) (EC 3.4.4.4)	1.2 ± 0.2	3.5 ± 0.2

Values are mean activity $\pm$ s.d. All data were determined for sparse cultures on 0.1% Triton X-100 homogenates of the cell lines^{9,16}. Proteolytic activity was determined using ³H-acetyl haemoglobin as substrate¹⁷. Glycosidase and acid phosphatase activity was determined using *p*-nitrophenyl derivatives as described^{9,16}.

Roseman¹¹ has postulated that cell surface glycosyl transferases and cell surface acceptors may be responsible for cell to cell adhesion; this postulate has gained some experimental support^{5,12-14}. Cell surface glycosyl ectoenzyme systems were measured in the present cells, and the results are given in Table 5. High levels of activity of surface glycosyl transferase: acceptor reactions were observed in the sparse line 37 cells compared to confluent cultures of both lines and to sparse cells of line 26. Thus the sparse cells of line 37 had much higher levels of surface glycosyl transferase: acceptor activities than line 26 growing either sparsely or at confluency. It should be noted that the high levels of surface enzymes and acceptors in the sparse cells of line 37 may enable them to adhere to surfaces of normal cells, such as platelets^{14,16}, which also have high levels of surface ectoenzymes or epithelial surfaces containing incomplete glycoproteins, thus contributing to movement of cells during implantation and metastasis. The high levels of surface glycoprotein: glycosyl transferases may contribute to cell

Table 5 Cell Surface Ecto-enzyme Glycosyl-Transferase: Acceptor Reactions

¹⁴ C-labelled precursor	Cell line			
	26	Sparse 37 (c.p.m. per 10 ⁶ cells)	26	Confluent 37
UDP-glucose	497 ± 57	802 ± 235 *	482 ± 61	490 ± 58
UDP-galactose	792 ± 73	2,906 ± 304 *	846 ± 78	929 ± 92
UDP-N-acetylglucosamine	846 ± 88	1,699 ± 108 *	808 ± 42	1,691 ± 104 *
UDP-N-acetylgalactosamine	1,000 ± 218	1,201 ± 192	1,042 ± 181	1,111 ± 162
GDP-fucose	186 ± 27	449 ± 90 *	451 ± 142	488 ± 59
GDP-mannose	802 ± 305	2,015 ± 264 *	912 ± 146	1,040 ± 84
CMP-N-acetylneuraminic acid	953 ± 106	3,423 ± 298 *	948 ± 140	1,410 ± 210

Experiments were performed by collecting cells at sparse state or at confluency by scraping, washing the cells once with 0.1 M Tris (pH 7.6), saline, suspending 0.1 to 0.3 mg as protein of the cells in 0.1 ml Dulbecco's medium without serum, adding 10 µl of 0.1 M MgCl₂, 10 µl of 0.1 M MnCl₂ and 10 µl of a 3.3 µCi ml⁻¹ solution of ¹⁴C-labelled precursor to a final volume of 0.130 ml. The mixtures were incubated for 30 min at 37° C, after which three volumes of 1% phosphotungstic acid in 0.5 N HCl were added and the precipitate was centrifuged out of solution. The precipitate was washed twice with 10% trichloroacetic acid and once with ethanol: diethyl ether (2:1, v/v) and dissolved in 1 N NaOH; radioactivity was determined in a liquid scintillation counter. Data are means ± 1 s.d. from independent observations.

* Significantly different from line 26 ($P < 0.05$).

movement within the circulation by adhering to normal circulating cell surface ectoenzyme systems.

We observed profound changes in the cell surface and in membrane synthetic and degradative enzymes during conditions of sparse growth of a cell line which showed high implantation. When cells were collected at confluency, the high implantation cell line more closely resembled the low implantation line in surface properties; since the cell lines have similar doubling times, cell growth does not seem to be a determinant. It is not known whether these same changes are favoured *in vivo* during periods of sparse growth or whether our findings are applicable to other tumour cell lines. From the data presented here, however, cell surface maturation (or its lack) seems to be a key factor in the cell's ability to implant. Finally, although the extreme differences between the high implantation and low implantation cells seen in the sparse cultures are of interest, the fact that confluent cells of both cell lines are similar has perhaps even greater importance. Even though the genome of line 37 must carry information that makes it have greater potential for implantation and information for extensive cell surface changes, these changes are minimised upon cell contact at confluency and the surface cell properties of cell line 37 become similar to those of cell line 26.

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Inhibition of Carcinogen Formation in Skin irradiated with Ultraviolet Light

THERE is no doubt that sunlight and ultraviolet light cause certain types of skin cancer^{1,2}. Unna first called attention to the high incidence of skin cancer among sailors exposed to sunlight, and his observation on the relationship between the development of skin cancers and exposure to sunlight has been confirmed by many others.

The mechanism of ultraviolet carcinogenesis, however, is not known. We have demonstrated that a mixture of cholesterol-derived photoproducts was present in both human^{3,4} and hairless mouse⁵ skin after exposure to ultraviolet light. Nanogram quantities of cholesterol-derived photoproducts were reported in human skin specimens irradiated with low doses of ultraviolet light. Furthermore, a compound with known carcinogenic properties, cholesterol α -oxide, was identified among those photoproducts. These findings lend support to the hypothesis that photochemical conversion of skin cholesterol to carcinogenic substances may play a role in the induction process of this actinic disease.

Vitamin E, vitamin C and some other biological antioxidants are known to reduce lipid peroxidation damage. Although their relationships are complex, non-biological relationships between antioxidant protective systems and oxidant-labile unsaturated compounds are well established and provide a valuable background for biological study. Here we provide evidence that the formation of cholesterol α -oxide can be inhibited in ultraviolet-irradiated skin by the

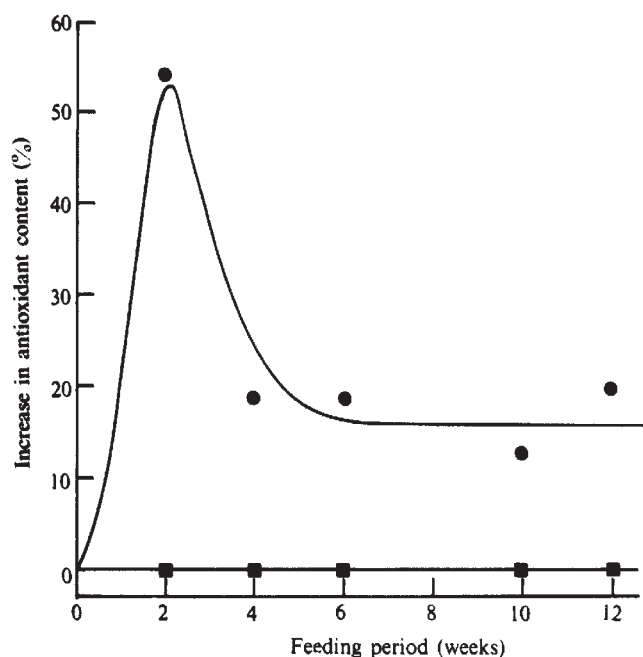


Fig. 1 A comparison of skin antioxidant content between controls and those animals receiving supplemental diets. ■, Controls contained approximately 1.46 μ equivalents antioxidant per g skin and served as baseline levels. ●, % of skin antioxidant levels of animals receiving supplemental diets.

dietary intake of antioxidants and, therefore, suggest a basis for preventive measures against ultraviolet-induced skin cancer.

Female hairless mice (Smith-Meyers strain) were divided into two groups. One group served as controls and received a regular balanced diet. The other group received the regular diet supplemented with the following additives: 1.2% (w/w) ascorbic acid; 0.2% D,L- α -tocopherol powder (250 IU vit E g⁻¹); 0.1% glutathione (reduced form) and 0.5% butylated hydroxytoluene. Skin was obtained from animals killed at intervals of 2 weeks. The skin specimens were incubated in the presence of 0.3 μ Ci of ³H-(1,2)-cholesterol (specific activity 52.6 Ci mmol⁻¹) suspended in Krebs-Ringer phosphate buffer, pH 7.4, for 18 h at 4° C. The specimens were then rinsed thoroughly to remove exogenous cholesterol and irradiated for 30 min in an environmental chamber maintained at 37° C in an ambient atmosphere. Control skin specimens were treated in an identical manner. Specimens were irradiated through a plane silica lens with a Burdick QA-450N mercury arc lamp (1.43×10^5 erg s⁻¹ cm⁻²).

After irradiation the specimens were homogenised in 10 ml deionised water with a Model 10 Polytron equipped with a saw-toothed generator. The total lipids were extracted with chloroform: methanol (2:1, v/v). Photo-oxidation products in the total lipid extracts were isolated by thin-layer chromatography (TLC). Quantitation of cholesterol α -oxide was accomplished by previously described methods⁸. Total water-soluble antioxidant content of each skin sample was determined spectrophotometrically by the method of Glavind⁹.

A comparison of skin antioxidant content between controls and those animals receiving supplemental diets for the experimental period can be seen in Fig. 1. Control animals contained an average of 1.46 μ equivalents of antioxidant per g of skin. The maximum increase (54%) in antioxidant content of those animals receiving the supplemental diet occurred in the first 2 weeks of feeding after which the level decreased and was then maintained at approximately 18% above controls.

The antioxidants used in these studies are all known to act

as free radical scavengers and to inhibit lipid peroxidation. The feeding of all four compounds ensured a wide spectrum of antioxidant activity. No gross morphological effects of the skin in those animals receiving the supplemental diet were observed, nor were there noticeable side effects in the animals.

In Fig. 2 it can be seen that the increase in antioxidant content of the skin provided protection against the formation of ultraviolet-induced cholesterol α -oxide. Complete protection against formation of this carcinogen was observed in those animals which had received the supplemental diet for 2 weeks. At this time the maximum increase in skin antioxidant content had occurred. Approximately 50% protection against formation of cholesterol α -oxide was afforded to those animals fed the supplemental diet for 4, 6, and 10 weeks.

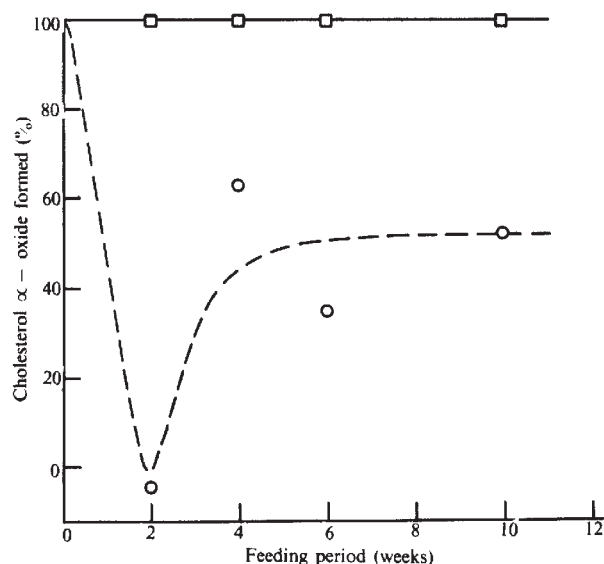


Fig. 2 Inhibition of ultraviolet-induced cholesterol α -oxide in skin. □, Levels of ultraviolet-induced skin cholesterol α -oxide in control specimens after 30 min of irradiation. These levels are represented as 100%. The yield of radiolabelled ultraviolet-induced cholesterol α -oxide after 30 min irradiation is equivalent to approximately 30 ng per g tissue⁸. ○, % ultraviolet-induced skin cholesterol α -oxide in those animals receiving supplemental diets as compared with controls.

Many protective measures against sunlight and ultraviolet light damage to skin are known. Sun screening agents have been demonstrated to reduce sunburn and skin cancer⁷, but many are far from ideal and there are few that are fully effective⁸⁻¹⁰. In the past numerous compounds have been suggested as possible effective systemic agents against adverse effects of sunlight. Most, however, show toxic effects and their practical systemic use is limited in the prevention of skin cancer. It is obvious that further investigation is needed in order to provide satisfactory protection.

We have now shown that antioxidants, known to impede lipid peroxidation, inhibit the photochemical conversion of skin cholesterol to cholesterol α -oxide. The role of this compound in ultraviolet-induced carcinogenesis is now under investigation¹¹. Moreover, our findings suggest the possible prophylactic effect of systemic antioxidants on the formation of this carcinogen and the subsequent pathological conditions which may result from its formation. Studies related to the prevention of light-induced skin cancer, contingent upon this hypothesis, are presently under way.

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Dietary Enhancement of Intestinal Carcinogenesis by Dimethylhydrazine in Rats

WHILE investigating the effect of marginal lipotrope deficiency in rats on chemical carcinogenesis, we have found an effect in an organ other than liver. We have already reported the enhancement of aflatoxin B₁-induction of hepatocarcinoma by lipotrope deficiency and recently found that N-nitrosodiethylamine and N-nitrosodibutylamine induce hepatocarcinoma earlier and in higher incidence in lipotrope-deficient rats than in normal rats¹⁻⁸. In the study reported here, induction of tumours of large and small intestine by symmetrical dimethylhydrazine (DMH) was examined in normal or lipotrope-deficient rats and was found to be enhanced in deficient rats.

Male, 4-week-old, Sprague-Dawley rats (Charles River Laboratories) were fed a semisynthetic diet which was either adequate in all known respects for rats (diet 1) or high in fat and marginally deficient in the lipotropes, choline and methionine (diet 2)⁹. After 3 weeks the rats were divided into four groups and given DMH intragastrically: (1) diet 1, DMH 30 mg kg⁻¹ per week for ten doses to twenty-eight rats; (2) diet 2, DMH 30 mg kg⁻¹ per week for ten doses to thirty-one rats; (3) diet 1, DMH 30 mg kg⁻¹ per week for five doses to twenty rats; (4) diet 2, DMH 30 mg kg⁻¹ per week for five doses to

nineteen rats. The five doses were given in 7 weeks and the ten doses in 14 weeks, the weekly dose being omitted in all groups in a week in which weight gain was poor in any one group. After treatment the rats were maintained on their respective diets until they began to bleed heavily from the rectum, developed large tumours of the ear canal, appeared moribund or died. Complete autopsy was performed; tissues were processed for histology by standard methods.

The marginal lipotrope deficiency supports normal or nearly normal weight gain and increases hepatic lipid content only slightly, to $11.3 \pm 1.1\%$ (s.e.) compared with the normal value of $7.5 \pm 0.2\%$. Deficient rats were more sensitive than normal rats to the toxicity of DMH and gained weight more slowly. They therefore received a smaller absolute total dose of the compound. Cumulative probability of death with intestinal tumour, a standardised method of comparing tumour death rates, was calculated from the autopsy data up to 34 weeks after DMH (Fig. 1)³. Both diet and dose of DMH influenced the length of time between the first dose of DMH and death with intestinal carcinoma. Deficient rats (diet 2) given the higher dose began to die with tumour 17 weeks after the initiation of treatment, and the curve rose steeply with time. Normal rats (diet 1) given the larger dose began to die with tumour at 21 weeks and also suffered a rapid attrition at an approximately 4-week interval behind the deficient rats. Average life span after the first dose of DMH was 172 ± 6 d (s.e.) for rats fed diet 2 and 195 ± 6 d for rats fed diet 1. The difference is statistically significant, $P < 0.02$. At the smaller dose, deficient rats began to die with tumour at 18 weeks, but the curve rose less steeply than in deficient rats given the higher dose. Normal rats began to die at 23 weeks. Average life span has not been significantly affected by diet in rats given the smaller dose because of the increased incidence of ear duct tumours in rats fed diet 1.

Total mortality up to 40 weeks after the first dose of DMH is given in Table 1. It was the result not only of intestinal tumours but also of ear duct tumours which occurred in greater incidence in the normal rats (Table 1). Of rats fed diet 1 and given the larger dose of DMH, 25% were killed because of signs of intestinal tumours and the remainder because they bore large ear duct tumours. In rats fed diet 2, 66% were killed because of signs of intestinal tumours. At the lower dose of DMH, 45% of rats fed diet 1 and 63% of rats fed diet 2 have been killed because of signs of intestinal tumour. Rats fed lipotrope-deficient diet had twice as many colon tumours per tumour-bearing animal as normal rats at both doses of DMH, but diet did not consistently affect the number of tumours of the small intestine.

Therefore, rats fed the deficient diet were more sensitive to both the toxicity of DMH and its carcinogenicity for the colon as measured by life span between DMH administration and death with intestinal carcinoma, cumulative probability of death with intestinal carcinoma or numbers of colon tumours per tumour-bearing animal. They were significantly less sensitive to DMH carcinogenesis in the ear duct. No significant effect of diet on carcinogenesis in the small intestine was found.

Table 1 Tumour Induction by Dimethylhydrazine in Normal or Lipotrope-deficient Rats

Diet	Total DMH (mg kg ⁻¹)	% Mortality*	% Rats dead with carcinoma of:			No. tumours per tumour-bearing rat in:	
			Colon†	Small intestine†	Ear duct‡	Colon	Small intestine
1	300	100	86	45	75	2.0	1.4
2	300	100	100	55	44	3.7	1.4
1	150	80	56	25	56	1.1	2.0
2	150	68	85	15	15	2.6	1.0

* 40 weeks after initial dose of DMH.

† Adenocarcinoma with varying degrees of differentiation and mucus production.

‡ Squamous carcinoma. Difference between rats fed diet 1 and diet 2 is significant if two doses are combined, $P < 0.05$.

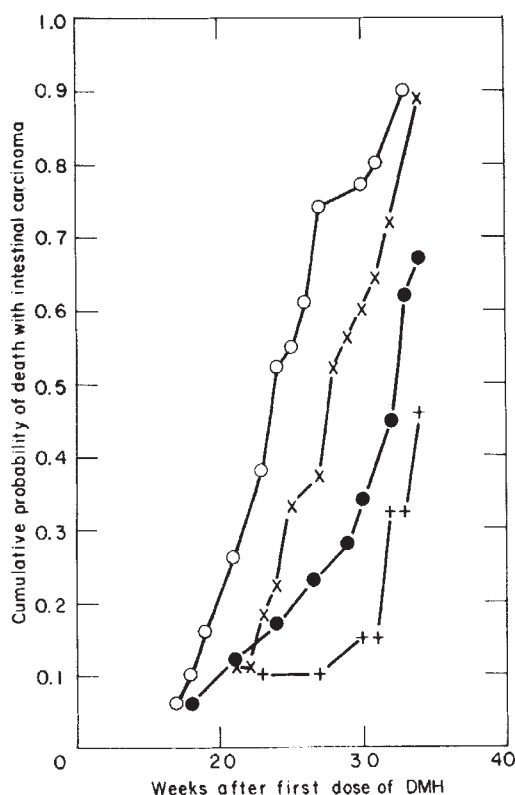


Fig. 1 Cumulative probability of death with intestinal carcinoma in rats fed either adequate diet (diet 1) and given DMH, 30 mg kg⁻¹ for either ten doses (x, twenty-eight rats) or five doses (+, twenty rats) or marginally lipotrope-deficient diet (diet 2) and given DMH, 30 mg kg⁻¹ for either ten doses (O, thirty-one rats) or five doses (●, nineteen rats).

This suggests that the dietary effect is exerted in the colon itself, either directly on the mucosa or indirectly by altering bacterial flora and its metabolism of the carcinogen.

Diet has been postulated to be responsible for the marked geographic variations in human colon carcinoma. Both dietary fat and carbohydrate have been incriminated^{4,5}. In experimental studies we have found that vitamin A deficiency enhances induction of colon carcinoma by aflatoxin B₁ but not by DMH^{6,7}. The study reported here has demonstrated enhancement of DMH induction of intestinal carcinoma by lipotrope deficiency. Whether the effect is due to the deficiency *per se* or to the high fat content of the diet used to induce it must be answered by further studies.

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"Asian Cigarette" is an Adverse Prognostic Factor in Peripheral Arterial Disease

In parts of South East Asia physicians believe that the strong, inexpensive locally made cigarettes are a risk factor in the development of peripheral arterial disease. Thus, in Ceylon the young men who smoke Beedi cigarettes are said to be particularly prone to occlusive diseases of the small arteries¹. A study of the Buerger syndrome in Korea revealed that the patients smoked a coarse form of locally grown tobacco². In other parts of Asia where the Buerger syndrome has been described the disease predominates in the lowest socio-economic groups (N. K. Yong, personal communication, and refs 3 and 4). Presumably, these poor people smoke the locally grown and locally manufactured cigarettes most often since they are the cheapest available.

In a recent epidemiological study of a large number of patients with the Buerger syndrome in Indonesia the patients were found to smoke cigarettes of three basic types⁵. The commonest was 'Kawung', the tobacco of which is grown, air cured and prepared at home by the patients or their neighbours. It is a coarse form of tobacco rolled in a tissue made from the leaves of the sugar palm and is the cheapest type of cigarette available in Indonesia. 'Kretek' cigarettes are made from a better quality, commercially available tobacco mixed with cloves and spices and wrapped in a normal tissue as used in Western cigarettes. 'Western'-type cigarettes were also smoked by these poor patients as were also conglomerates of tobacco removed from discarded cigarette butts and remanufactured by some enterprising merchant.

Because there was no information available about the smoking habits of the controls at a time comparable to the onset of the disease in the patients they were matched with, we are not able to comment on the Kawung cigarette as a risk factor.

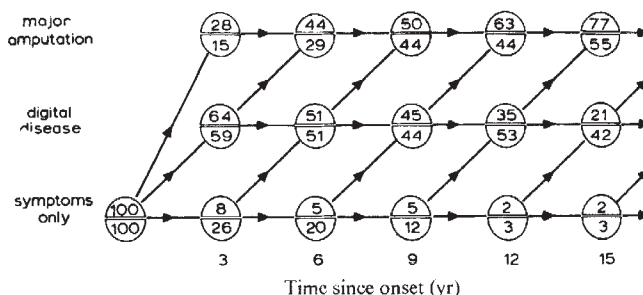


Fig. 1 State flow chart showing the expected clinical course of Buerger's disease in 100 patients who smoked Kawung cigarettes (upper half circles) compared with the expected clinical course in 100 patients who did not smoke Kawung cigarettes (lower half circles).

In Fig. 1, however, a state flow chart describes the expected clinical course for 100 Indonesian patients with Buerger's disease who smoked Kawung cigarettes, and compares it with the expected clinical course for 100 patients who smoked Kretek cigarettes, Western cigarettes or a combination of these two. At each time interval, during the course of the disease for the group of patients, Kawung cigarettes are associated with a worse prognosis. The state flow chart is derived from a mathematical model of Buerger's disease and the theoretical aspects are detailed elsewhere^{6,7}.

Although we found that Kawung smoking was associated with a low socio-economic status, which in itself was found to be an adverse prognostic factor, it was also found that Kawung smokers of higher socio-economic status had just as poor a prognosis as Kawung smokers of low socio-economic status.

Furthermore Kawung smokers were not found to be heavier smokers than the other patients⁷.

These findings are in accord with the opinion of Indonesian physicians who feel that the cheap Kawung cigarette is both an important risk factor and an important prognostic factor for Buerger's disease. Our findings also lend weight to the clinical impression of doctors throughout South East Asia that local cheap home-made cigarettes are often associated with the development of peripheral vascular disease and also with a worse prognosis than Western-type cigarettes.

We suggest that it may be of some value to examine more closely the Asian cigarette and especially the Indonesian Kawung cigarette which has been shown to be directly associated with a worse prognosis in at least one type of peripheral vascular disease. More epidemiological and clinical data are needed as well as direct information about the cigarettes themselves. As far as we are aware the nicotine content of the Kawung cigarette is unknown and carboxyhaemoglobin levels in Kawung smokers have not been studied.

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Antibodies to α -Foetoprotein cause Foetal Mortality in Rabbits

α -FOETOPROTEINS (AFP) have been observed in the serum of all mammalian species so far studied^{1,2}. In the rabbit AFP constitutes about 33% of the total serum protein on day 20 of gestation and reaches a peak concentration around days 24 to 26 (ref. 3). This ontogenic pattern is similar to that of the rat where maximum concentration is also reached late in gestation, but differs from the human where a steady decline occurs after the first trimester^{4,5}. Although considerable interest has attached to the reappearance of AFP in the serum of adult animals and human patients with hepatocellular cancer⁶⁻⁹, little attention has been given to the possible role of this protein in normal development. The preliminary experiments reported here were designed to investigate this aspect by selectively neutralising AFP *in vivo* with specific antibodies.

Dutch rabbits in their first pregnancy were injected intravenously on the twenty-first day of gestation with 2 ml per kg body weight of high titre sheep anti-AFP¹⁰; control animals received similar doses of normal sheep serum. The animals were killed 24 h later and the condition of the foetuses was examined. The results are summarised in Table 1.

There was a very high incidence of foetal mortality in animals injected with anti-AFP. The extraembryonic fluids of the dead foetuses were invariably contaminated with blood, and the yolk sac was fragile and translucent showing no vitelline circulation. Most of the foetuses that were

Table 1 Foetal Mortality

Rabbit	No. of foetuses		No. dead		%
	Left	Right	Left	Right	
1	4	4	4	4	100
2	8	3	5	2	63.5
3	2	7	0	2	22
4	2	4	2	4	100
5	1	4	1	1	40
C ¹	4	3	0	0	0
C ²	2	2	0	0	0

C¹ and C² were controls.

alive looked healthy although one or two showed signs of superficial haemorrhage. Analysis of the uterine fluid revealed the presence of sheep antibodies. These were also present in the exocoelomic fluid of both live and dead foetuses, appreciably more being present in the latter. The amniotic and allantoic fluids and the foetal serum were consistently negative for sheep antibodies in both live and dead foetuses.

Previous studies have shown the importance of the yolk sac as a primary site of AFP synthesis in the rabbit^{10,11} and in the rat¹² and human¹³. It is also well established that the yolk sac plays an important role in the transmission of maternal antibodies to the foetus in rabbit and rodents¹⁴, as well as subserving other important functions during development¹⁵. In view of the fact that sheep antibodies are poorly transmitted to the rabbit foetus¹⁴ and that no evidence could be found for their presence in the foetal serum here, it seems unlikely that the anti-AFP was exerting its effect on the embryo directly, although this possibility cannot be entirely discounted. It seems more probable that the site of action was the yolk sac, but whether foetal death resulted from a specific deficiency of AFP or a more general impairment of yolk sac function cannot as yet be concluded. The fact that some foetuses survived apparently unharmed presumably reflects either a differential exposure to the antibody or variation in susceptibility to its action. Studies are currently in progress to try to resolve these problems.

Antibodies directed towards the yolk sac are known to produce congenital malformations and foetal mortality in pregnant rats¹⁶, and *in vitro* studies have demonstrated that the primary action is on the visceral yolk sac rather than on the embryo directly and is independent of any immunological reaction of the mother¹⁷. A similar explanation would seem appropriate for the results presented here; indeed, it is not improbable that at least one of the active components in yolk sac antiserum is directed towards AFP.

Antibodies to AFP clearly have disastrous effects on the foetus and it is relevant to speculate whether some congenital malformations and abortions may be due to maternal isoimmunisation.

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Serum Progesterone and Luteinising Hormone Levels in Non-suckled Primiparous Heifers

PRIMIPAROUS heifers normally have a longer interval from parturition to oestrus, ovulation and subsequent conception than do mature cows. Lactation also extends this interval. We have therefore measured simultaneously luteinising hormone (LH) and individual progesterone within the same serum sample throughout the postpartum interval in the bovine species in order to elucidate the endocrine events that occur before the first oestrus.

In Fig. 1, the horizontal scale from -24 reveals a similar ovarian and endocrine pattern preceding oestrus for the three non-suckled primiparous heifers. The interval from parturition to first oestrus for heifers 1, 2 and 3 (Fig. 1) was 22.75, 27.75 and 35.75 d, respectively.

Cyclic follicular activity was recorded throughout the postpartum interval for each heifer and follicular growth was intensified for several days preceding oestrus. Ovulation with subsequent corpus luteum formation occurred on the left, right and right ovaries, respectively, for heifers 1, 2 and 3.

Increased levels of LH were observed for the length of approximately one cycle (day -17 to day -20) before oestrus; thereafter, LH remained at low levels until oestrus. A typical LH peak coincided with oestrus for all three heifers, reaching 13.3, 11.0 and 65.0 ng ml⁻¹, respectively. The LH peak observed at oestrus agrees with other reports (ref. 6 and personal communication from J. N. Wiltbank).

Sustained 4-pregnene-3,20-dione (P) and 20 β -hydroxy-4-pregnen-3-one (20 β -OHP) release from undetectable levels occurred several days before oestrus for all three heifers. The increased levels of P and 20 β -OHP preceding oestrus differed among the three heifers as to duration of release and levels.

Auxiliary peaks of 17-hydroxy-4-pregnene-3,20-dione (17-OHP) were observed before the major P and 20 β -OHP release for heifers 1 and 3. In all three heifers, increased levels of 17-OHP occurred during the time of increased serum P and 20 β -OHP release. A significant 17-OHP peak occurred at or near oestrus for all three heifers. Not until both P and 20 β -OHP had reached a very low serum level before oestrus did the 17-OHP and LH peak occur. It is interesting that a second 17-OHP peak occurred after oestrus, near ovulation, for heifer 3. This 17-OHP peak near ovulation has also been observed for the heifer exhibiting a normal oestrus cycle (unpublished results of R. L. T., D. T. Atkins and J. L. F.).

Plasma progesterone levels of approximately 1 ng ml⁻¹ have been reported in the postpartum cow, with a level of slightly over 2 ng ml⁻¹ 7 d before first oestrus (ref. 6 and personal communication from J. N. Wiltbank). Some workers have reported increased elevation of P levels 3 to 5 d before the first postpartum oestrus⁷, whereas others found postpartum plasma P levels to be present in a cyclic pattern within 20 d postpartum, similar to that observed for non-pregnant mature cows⁸. In two cows in which the P analysis was

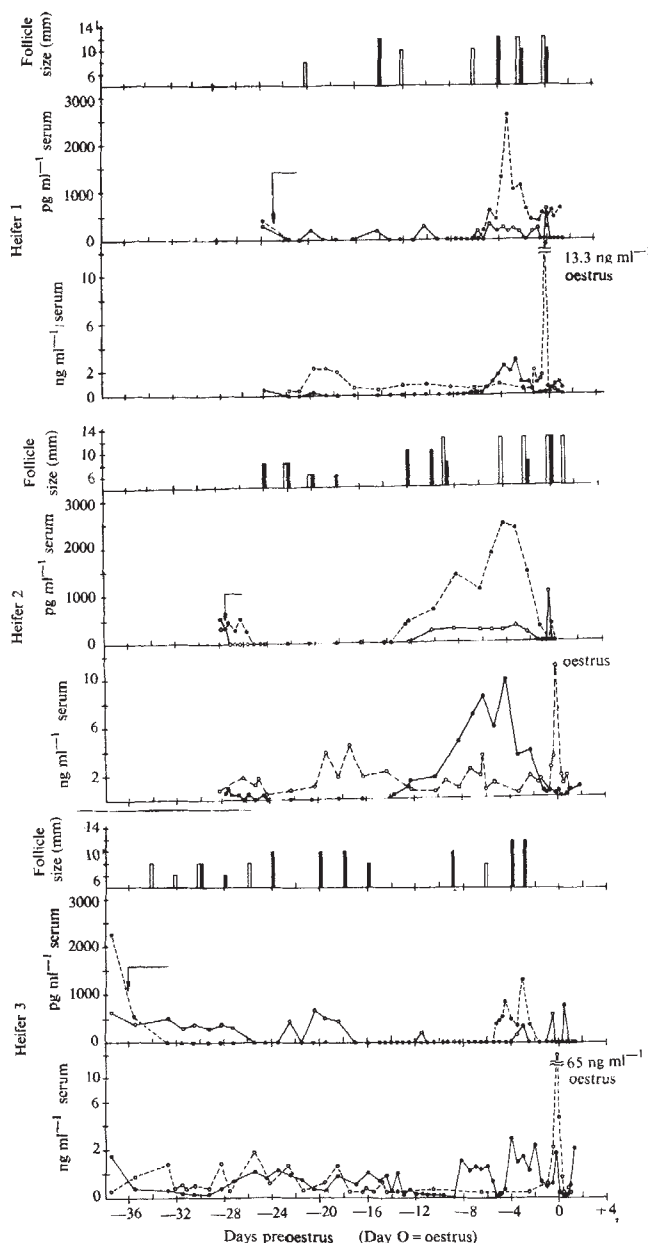


Fig. 1 Results obtained from three non-suckled Hereford heifers (1, 2 and 3) from parturition to first oestrus postpartum. Indwelling jugular cannulas were inserted into these heifers approximately 2 weeks before calving to expedite procuring multiple blood samples. Samples of 20 ml of blood were collected at intervals of 6 h through parturition to the first postpartum oestrus. At each of these blood collections, oestrus was detected with the aid of a penectomised bull. The blood was allowed to clot at room temperature and the serum was frozen. Blood cellular components were monitored periodically to determine the health of the animal. Calves were removed from these heifers immediately after birth. All heifers were palpated every second day to determine ovarian activity and gross uterine involution rate. Undetectable levels of the hormones are plotted on the base line. Note that both 20 β -hydroxy-4-pregnen-3-one (20 β -OHP) and 17-hydroxy-4-pregnene-3,20-dione (17-OHP) are in units of pg ml⁻¹, whereas LH and 4-pregnene-3,20-dione (P) are in units of ng ml⁻¹. LH was radioimmunoassayed by the double-antibody technique¹ using bovine LH (NIH-LH-B8) as the standard. Both P and 17-OHP were measured by radioimmunoassay using an antibody directed against 11-deoxycortisol^{2,3}. Serum (1 ml) was extracted with ether and P, 20 β -OHP and 17-OHP were separated on a micro-Celite column⁴. The 20 β -OHP was quantitated as P after enzymatic oxidation⁵ and resubmission to the 'Celite' column. Black columns, right ovary; white columns, left ovary. For each heifer the upper graphs represent: ●---●, 20 β -OHP; ○---○, 17-OHP; and the lower graphs represent: ●---●, P; ○---○, LH (NIH-LH-B8). The arrows indicate parturition.

carried to the first postpartum oestrus, an increase in P from approximately 0.20 ng ml^{-1} to a peak of approximately 1 ng ml^{-1} preceded oestrus by a few days⁸. Echterkamp⁹ found in lactating Holstein cows that plasma P values remained at low basal levels (0.0 to 0.3 ng ml^{-1}) until the first oestrus postpartum. Robertson¹⁰ reported that auxiliary plasma P peaks occurred before the first postpartum mucous discharge in cows and was uncertain whether these P peaks were preceded by ovulation or whether they were derived from luteinised follicles.

The "two cell theory", described in the bovine species^{11,12}, postulates that the theca interna has the enzymes to convert P to oestrogen which is representative of follicular activity, with no apparent 20β -hydroxysteroid dehydrogenase (20β -HSD), whereas other workers¹³ have found that 20β -HSD activity resides in both the granulosa and theca cell layers. The luteinised granulosa cell which is representative of luteal activity lacks the 17 -hydroxylating enzyme, but has 20β -HSD¹¹⁻¹³. Other researchers have confirmed the findings observed for the "two cell theory", isolating P and 17 -OHP in follicular fluid¹⁴, whereas only P and 20β -OHP were found in luteal tissue¹⁵ with very little, if any, ability to aromatise C-19 steroid precursors into oestrogens¹⁶.

In our study, increased LH levels were observed before the onset of P release and the P levels and time duration data follow a pattern similar to the mature cow. The facts that neither oestrus, ovulation nor corpus luteum formation was observed before P release, and that 20β -OHP was present, suggest that a follicle was luteinised. Others^{13,17} found with the bovine species that luteinisation of both theca and granulosa cells of preovulatory follicles began before ovulation. LH can cause pseudoluteinisation of non-ovulatory follicles and (or) cause ovulation of mature follicles in humans¹⁹. The granulosa cell layer of luteinised follicles from rabbits synthesises as much P as do the corpora lutea of pseudopregnant rabbits²⁰. The oocyte itself and (or) high levels of LH induce luteinisation, which determines the specific steroid hormones produced in the rat²¹.

We believe that this is the first report of monitoring of bovine blood levels of 17 -OHP. High levels have been found in follicular fluid, but not in the corpus luteum or ovarian stroma¹⁴. The presence of the peak 17 -OHP observed before ovulation for the three heifers suggests that 17 -OHP serves a role similar to that observed in women, where ovarian venous²² and peripheral plasma levels^{23,24} of 17 -OHP begin to increase 3 d before ovulation and peak coincident with the mid-cycle LH peak. The 17 -OHP level has been shown to be the highest in the human ovary bearing the mature follicle²². It has been postulated that both oestradiol- 17β and 17 -OHP have to be present in order to achieve the mid-cycle surge of gonadotrophins in women necessary for ovulation²⁵. Likewise, an increase in 17 -OHP and oestradiol- 17β has been reported in equine follicular fluid just before ovulation²⁶. A pharmacological dose (20 mg per cow) of 17 -OHP injected into ovariectomised dairy heifers, however, had no detectable effect on LH levels²⁷.

Low levels of P seem to be necessary for LH synthesis and (or) release in the bovine female²⁸. Investigators have postulated that in the human female, constant, slowly changing, or high levels of P inhibit both tonic and surge LH, while low, rapidly increasing levels have a net stimulatory effect^{18,29}.

Our preliminary findings on the endocrine events preceding the re-establishment of normal cyclic activity in three non-suckled postpartum heifers demonstrate increased LH levels 17 to 20 d before oestrus, which is succeeded by a dramatic synthesis and (or) release of P and 20β -OHP for several days preceding the LH peak at the time of oestrus. At present, no specific role of 20β -OHP has been identified for the bovine female. There is strong evidence that its α -epimer, 20α -hydroxy-4-pregnen-3-one, acts as a positive feedback mechanism for LH discharge in the rat³⁰ and rabbit³¹.

Perhaps 20β -OHP is required to return the biological system to its normal cyclic pattern.

An improved understanding of the endocrinological changes associated with the postpartum interval should be beneficial in formulating means of decreasing this interval, thereby allowing an increase in the life-time production of the cow. Although our data in this communication are derived from three heifers, we are confident that the result is a common phenomenon.

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Is Cortisol Responsible for Inhibition of MLC Reactions by Pregnancy Plasma?

THE foetus inherits histocompatibility antigens from the father and becomes an allograft to the mother, but is not rejected. Possible reasons for this have been discussed by Billingham¹. Various hypotheses, including a physiological barrier between the mother and the foetus and a reduction of the immunological reactivity of the mother during pregnancy, have been suggested. I recently reported the inhibitory effect of pregnancy plasma on the mixed leukocyte culture (MLC), which I believe not to be mediated by an antibody but possibly by the particular hormonal situation of pregnancy². Here I report experiments to study whether 17-hydroxycorticosteroids are inhibitory factors in pregnancy plasma. I studied the inhibitory activity on the MLC reactivity of plasma taken at various intervals during pregnancy and measured the plasma cortisol level of each specimen.

Blood samples were obtained from fifteen primiparous mothers immediately after delivery and from fifty-five primigravidas at the tenth and thirty-ninth weeks of gestation. Control plasmas were obtained from healthy adult females who had no history of pregnancy or blood transfusion. Cell-free plasmas were prepared from these heparinised blood samples (preservative-free heparin), 200 units per 10 ml blood) and frozen at -20°C until tested.

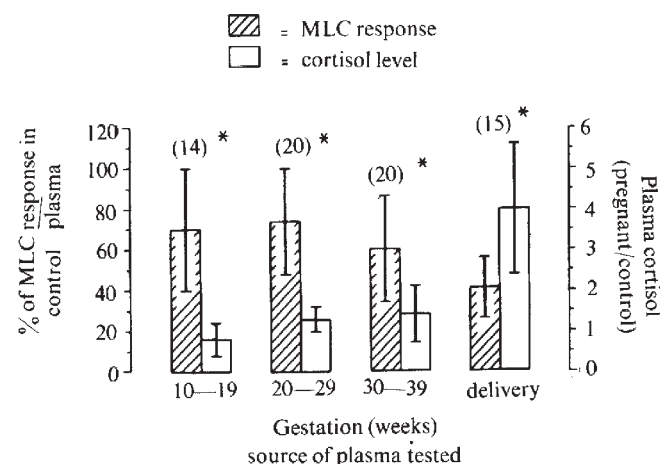


Fig. 1 Inhibitory activity of plasmas from primigravidas at various weeks of gestation and level of cortisol in these plasmas. In each experiment, five aliquots of MLC were prepared from a given pair. Each aliquot was suspended separately in test plasma of each group and in control plasma. Reactivity (c.p.m.) of MLC suspended in each test plasma is expressed as percentage of MLC reactivity in control plasma. The level of cortisol is expressed as the ratio of cortisol concentration of test plasma to that of control plasma. Mean values and s.d. are depicted.

* = Number of experiments in each group.

Leukocyte suspensions were prepared from normal volunteers, unrelated to one another. Preparation of leukocyte cultures and measurement of the incorporation of ^3H -thymidine into DNA have been described before². Donor leukocytes were resuspended in frozen stored pregnancy and control plasmas. The plasma containing the leukocytes was diluted with medium 199 so that the final plasma concentration was 20%. The cell suspensions were incubated in 17×100 mm disposable plastic tubes. The mixed cultures contained a 1:1 mixture of cells from two subjects in test pregnancy and control plasmas. The cultures were incubated at 37°C for 7 d. At the end of the incubation period, ^3H -thymidine (specific activity 5.0 Ci mmol^{-1}) was added to each tube to give a concentration of $1.0 \mu\text{Ci ml}^{-1}$. The

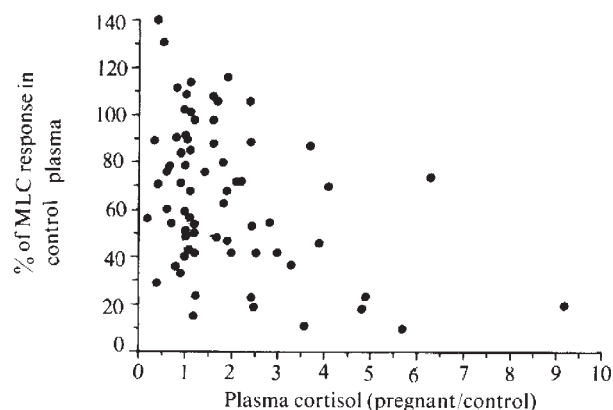


Fig. 2 Relationship of the inhibitory activity to the level of cortisol in the plasma of primigravidas, expressed as in Fig. 1. Dots represent the individual samples.

amount of ^3H -thymidine taken up was measured after incubation for 4 h.

The plasma cortisol level was determined by using the steroid-binding properties of cortisol-binding globulins³. In this study the range of cortisol levels in control plasma was $5\text{--}29 \mu\text{g per } 100 \text{ ml plasma}$.

Figures 1 and 2, and Table 1, show the relationship of inhibitory activity of pregnancy plasmas on the MLC reactivity to concentration of cortisol in these plasmas. Table 1 shows the results from five experiments, typical of the fourteen experiments performed. In each experiment, six aliquots of MLC were prepared from a given pair. Each aliquot was suspended separately in plasmas from six females, five of whom were pregnant. This experiment was repeated a total of fourteen times, always with different leukocytes and plasmas. As shown previously², most pregnancy plasmas were inhibitory to the reactivity of MLC. Their inhibitory activity was maximal at the time of delivery. The level of cortisol in pregnancy plasma rose during pregnancy and reached a maximum at the time of delivery.

Table 1 Effect of Pregnancy Plasma on Mixed Leukocyte Reaction

Experiment No.	^3H -thymidine uptake in cultures of mixed leukocytes from unrelated donors					
	Control plasma *	Pregnancy plasma † (gestation in weeks)				
		10-19	20-29	30-34	35-39	Delivery
1	27,388† (22.0)§	15,660 (13.9)	12,579 (21.6)	12,806 (28.8)	9,051 (35.2)	3,492 (65.3)
2	23,529 (17.0)	24,821 (9.0)	18,949 (33.1)	16,334 (40.9)	19,443 (36.5)	14,389 (69.3)
3	46,220 (17.4)	30,808 (21.3)	21,801 (33.5)	19,315 (31.8)	16,845 (30.8)	15,632 (50.8)
4	20,181 (22.5)	15,503 (18.4)	9,213 (25.0)	10,659 (22.9)	12,079 (57.0)	3,736 (81.3)
5	27,667 (12.0)	25,396 (20.4)	18,969 (25.4)	19,642 (26.3)	8,736 (52.5)	11,642 (75.0)

* Control plasmas were obtained from healthy adult females who had no previous history of pregnancy or blood transfusion.

† Pregnancy plasmas were obtained from primigravidas at various weeks of gestation and after delivery. Different plasmas were tested in different experiments.

‡ C.p.m. per culture; mean from triplicate cultures.

§ Plasma cortisol level ($\mu\text{g per } 100 \text{ ml plasma}$).

The mean values of inhibitory activity and cortisol level shown in Fig. 1 for each period of pregnancy suggested a correlation between inhibitory activity of pregnancy plasmas and the concentration of cortisol in these plasmas. Plotted as individual points (Fig. 2), there was no exact correlation between the inhibitory activity and cortisol content of each

pregnancy plasma. Within each experiment the pregnancy plasmas which contained the highest levels of cortisol were not necessarily the most inhibitory to the MLC reactivity. A correlation analysis was done for the data to examine the crude and partial correlations between MLC reactivity (c.p.m.), period of gestation (in weeks: non-pregnant control at zero time), and cortisol concentration (μg per 100 ml plasma). The data from each experiment were treated separately. The correlation coefficient for MLC inhibition and period of gestation was highly significant ($r = -0.63$; $P < 0.001$). The correlation coefficient for MLC inhibition and cortisol concentration was also highly significant ($r = -0.49$; $P < 0.001$). Since cortisol concentration was closely correlated with gestation time ($r = -0.61$; $P < 0.001$), a partial correlation analysis was, however, done to eliminate the effect of gestation time. When this was done, the individual partial correlations of MLC inhibition with cortisol concentration were inconsistent (ten negative; four positive), and the pooled coefficient was not significantly different from zero ($r = -0.18$; $P > 0.1$). Thus it seems that although the period of gestation is correlated with the degree of MLC inhibition, there is no close correlation between cortisol concentration and MLC inhibition.

Table 2 Effect of Temperature (60°C) on Inhibitory Activity of Pregnancy Plasma

Experiment No.	^3H -thymidine uptake ($\times 10^{-3}$) in mixed leukocyte cultures* containing		Incubated at 60°C for 1 h	
	Untreated Control plasma	Pregnancy plasma†	Control plasma	Pregnancy plasma
1	$34.3 \pm 4.9 \ddagger$	21.0 ± 5.6 (61%)§	17.0 ± 4.8	8.4 ± 1.8 (49%)
2	21.5 ± 1.6	8.4 ± 1.5 (39%)	23.5 ± 1.7	9.4 ± 1.6 (40%)
3	27.9 ± 2.2	6.9 ± 1.4 (25%)	23.1 ± 6.5	6.6 ± 0.6 (28%)
4	27.7 ± 1.7	11.6 ± 3.4 (42%)	20.6 ± 2.4	8.2 ± 1.2 (40%)
5	18.6 ± 2.2	8.4 ± 2.7 (46%)	16.4 ± 1.0	6.9 ± 1.7 (42%)

* In different experiments, mixed leukocyte cultures were prepared using different pairs of unrelated donors.

† Plasmas from primiparas immediately after delivery. Different plasmas were tested in different experiments.

‡ C.p.m. per culture; mean from triplicate cultures $\pm$ s.d.

§ Figures in parentheses indicate a percentage of the reactivity of MLC in control plasma.

Table 2 shows the effect of temperature (60°C) on the inhibitory activity of pregnancy plasma on the reactivity of MLC. Incubating pregnancy plasma at 60°C for 1 h had no effect on its inhibitory activity.

I have thus shown that cortisol is not a major inhibitory factor in pregnancy plasma. Unbound cortisol is considered to be the biologically active portion of the total plasma cortisol⁴, but reliable techniques are not readily available for measuring unbound cortisol. Therefore, I measured the total plasma cortisol. A parallel increase in the concentration of total plasma cortisol and its unbound fraction during pregnancy has been shown⁴.

At present, the nature of this inhibitory factor in pregnancy plasma is unknown. Ceppellini⁵ and Leventhal *et al.*⁶ have shown that an antibody is present in multigravida female plasma that is directed against HL-A specificities of stimulating cells and decreases their capacity to stimulate in MLC. Our inhibitory factor is unlikely to be an antibody against HL-A specificities for the following reasons: first, in this study, none of the test pregnancy plasmas were cytotoxic toward donor lymphocytes used in MLC by conventional assay techniques using rabbit serum as a source of complement⁷. Second, most pregnancy plasmas tested

inhibited the reactivity of MLC from unrelated pairs that were selected at random in each experiment.

Gatti *et al.*⁸ have found an inhibitory factor of MLC reactions in serum from a healthy multigravida female. Their factor contains the 7S IgG fraction and has the broad specificities, but it is not lymphocytotoxic or directed against HL-A specificities. While our inhibitory factor disappears shortly after termination of pregnancy², in their study inhibitory activity persists in the donor's serum 16 months after delivery of her last child⁸.

Stimson⁹ has suggested that a serum macroglobulin present in pregnancy sera (pregnancy-associated globulin) may be an immunosuppressive globulin. Horne *et al.*¹⁰ have, however, been unable to demonstrate that sera containing measurable quantities of this globulin have a depressive effect on the mitogenic response following phytohaemagglutinin (PHA) or tuberculin purified protein derivative (PPD) stimulation of lymphocytes. My inhibitory factor differs from this globulin. While this globulin is completely inactivated by heating the positive serum at 60°C for 10 min¹¹, my inhibitory factor is resistant to incubation at 60°C for 1 h (Table 2). I think that, rather than an antibody, progesterone, oestrogen and/or human chorionic somatomammotrophin (HCS) may be responsible for inhibitory activity. Progesterone and oestrogens show immunosuppressive properties as measured by prolonged allograft survival^{12,13}. It has also been shown that HCS suppresses PHA-induced lymphocyte transformation¹⁴.

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Animal Model to Study the Relationship between Immunoreactive Gastrin and Inflammatory Arthritis

THE observation of raised concentrations of radioimmuno-reactive gastrin in the plasma of patients with rheumatoid arthritis¹ has directed attention to the role of this foregut hormone in the inflammatory response. Evaluation

of the significance of this relationship would be simplified if a suitable animal model were available. We have studied immunoreactive gastrin in rats following the induction of adjuvant arthritis in an attempt to establish such a model.

A series of twenty-two adult male Sprague-Dawley rats was studied. Adjuvant arthritis was induced by subplantar injection of 0.5 mg killed *Mycobacterium butyricum* in 0.1 ml heavy mineral oil (adjuvant) into the left hind paw. The progress and severity of the arthritis were monitored by paw scores and by weight reduction in the affected animals. These assessments were carried out immediately before letting blood for gastrin assay on the day before injection of adjuvant and on the fourteenth day following injection.

Plasma gastrin was measured in venous blood samples, using a sensitive and specific radioimmunoassay following a 15 h overnight fast. This assay is based on antibody raised in rabbits to synthetic human gastrin. Labelled hormone is prepared using a modification of the chloramine-T method of Hunter and Greenwood² and separation of the free hormone from that bound to antibody is achieved with dextran-coated charcoal³. Precision of this assay is greatest below 600 pg ml⁻¹ and the lowest concentration of gastrin which can be detected is of the order of 10 pg ml⁻¹. Cross-reaction with cholecystokinin and pancreozymin is minimal and preliminary studies indicate that the antibody recognises not only the heptadecapeptide hormone but also the 'big' gastrin described by Yalow and Berson⁴.

The mean weight of the seventeen normal rats was 306.6 ± 5.1 g (s.e.m.) and of the thirteen injected rats was 293.8 ± 7.2 g (s.e.m.).

The mean radioimmunoactive gastrin level in the untreated group was 234.6 ± 35.9 pg ml⁻¹ (s.e.m.) and that of the adjuvant group was 401.4 ± 41.7 pg ml⁻¹ (s.e.m.). The difference between the two groups is statistically significant ($t=2.91$; $0.01 > P > 0.001$).

Table 1 Weight, Paw Scores and Gastrin Concentrations in Rats Before and After Injections of Adjuvant

Rat	Pre-adjuvant			Post-adjuvant		
	Weight (g)	Paw score (0-5)	Gastrin (pg ml ⁻¹)	Weight (g)	Paw score (0-5)	Gastrin (pg ml ⁻¹)
6	300	0	120	275	3	505
8	300	0	405	250	2	455
10	355	0	130	325	3	790
13	325	0	355	300	5	470
16	295	0	245	250	2	610
17	310	0	135	275	1	610
19	310	0	215	270	2	230
20	300	0	90	325	1	160
Mean	311.8		211.8	283.7		478.7
± s.e.m.	± 7.0		± 41.1	± 10.5		± 72.7

In eight rats it was possible to measure immunoreactive gastrin both before and after the successful induction of adjuvant arthritis (Table 1). The initial mean weight of this group of rats was 311.8 ± 7.09 (s.e.m.) and the weight of all but one of these animals dropped after adjuvant administration to a mean of 283 ± 10.5 g (s.e.m.). This drop is statistically significant ($t=3.415$; $0.02 > P > 0.01$). The initial mean gastrin concentration of this group was 211.8 ± 41.1 pg ml⁻¹ (s.e.m.). Fasting gastrin rose in all animals following adjuvant to a mean value of 478.7 ± 27 pg ml⁻¹ (s.e.m.). This rise is also statistically significant ($t=3.182$; $0.05 > P > 0.02$). It seems clear that adjuvant arthritis in rats is associated with an elevation of fasting plasma immunoreactive gastrin, providing a suitable model for further work on the significance of this hormone in the inflammatory process.

One criticism of the observation of raised serum immunoreactive gastrin in patients with rheumatoid arthritis¹ arose from the very real possibility that the phenomenon was produced by the drugs which the patients were receiving. In the light of our present results from untreated adjuvant rats it now seems unlikely that high gastrin levels are a consequence of drug therapy.

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IQ and ABO Blood Groups

THREE methods are available for the genetic analysis of continuous variables in human populations. One is the biometrical approach¹ which, in addition to partitioning the phenotypic variance into genetic and environmental components, has been used to reveal sex linkage² and provide estimates of the number of effective factors³. Second, genetic markers can be studied for evidence of pleiotropic effects⁴, and third, linkages between polygenic factors and segregating genetic markers can be sought^{5,6}. It is not always possible to distinguish between linkage and pleiotropy, so the second and third methods need similar data and thus go together.

In spite of the availability of segregating genetic markers, techniques for the location of polygenes⁷, developed in *Drosophila*⁸ but also very successful in mice⁹ and wheat¹⁰, have not been applied to human material. We are attempting to do so, and as a result have found an association between IQ and ABO blood group phenotypes. We have data for more continuous variables and more marker genes but the association reported here comes from the first system we have analysed.

The sample population comprised males and females between the ages of 18 and 70 living in six ecclesiastical parishes in Otmoor, a region some 5 to 10 miles north-east of Oxford¹¹. Age corrected IQs, measured on the Wechsler Adult Intelligence Scale¹², and ABO blood group phenotypes are available for 534 individuals. The sample was collected in seven villages on a house-to-house basis and contains some individuals known to be related to one another.

The data (Table 1) show significant differences in mean IQ between some of the ABO phenotypes. In particular the A₂ group has the highest mean IQ and the A₂ and the O phenotypes each have significantly higher mean IQs than the A₁ phenotype.

Table 1 Mean IQs of the ABO Phenotypes

	A ₁	A ₂	B	O	A ₁ B	A ₂ B
<i>n</i>	263	25	43	271	12	6
mean IQ	106.95	111.16	107.25	109.75	107.58	111.16
s.e.m.	0.72	2.01	1.86	0.67	2.21	5.2

The difference in mean IQ between the O and A₁ phenotypes observed overall is significant in the data from four of the seven villages (Table 2). Of the other three there is no difference in Beckley, but Horton, which is characterised by the highest mean IQ, seems to stand out because the direction of the difference is the other way round. This is not, however, significant. Further, the data for the seven villages provided no statistical evidence for heterogeneity in the differences in mean IQ between the O and A₁ phenotypes ($\chi^2_6 = 11.3$, $P > 0.05$). Unfortunately the number of A₂ phenotypes in the sample is too small to allow comparisons to be made between the villages.

Table 2 Mean IQs of the A₁ and O Phenotypes in the Seven Villages

	A ₁	O	Difference in mean IQ between A ₁ and O	Probability
Horton	115.89 (46)*	111.51 (47)	-4.38	$t_{91} = 1.6$ NS
Merton	107.71 (28)	112.57 (37)	+4.86	$t_{65} = 2.1$ 0.05
Beckley	109.68 (38)	109.60 (42)	-0.08	$t_{78} = 0.04$ NS
Wendlebury	105.41 (49)	111.64 (50)	+6.23	$t_{97} = 2.4$ 0.05 > $P > 0.01$
Weston	104.09 (32)	108.28 (29)	+4.19	$t_{59} = 1.98$ 0.05
Charlton	101.16 (57)	106.00 (55)	+4.84	$t_{110} = 2.4$ 0.05 > $P > 0.01$
Boarstall	103.92 (13)	107.82 (11)	+3.90	$t_{22} = 0.81$ NS

* Sample sizes given in parentheses.
NS = not significant.

Charlton was the first village for which complete data were available and analysed and they showed a significant difference in mean IQ between the O and A₁ phenotypes. As associations of this kind are notoriously suspect as statistical artefacts it was prudent to use the Charlton data to provide a hypothesis which could be tested by the data obtained from the six other villages, which were collected and analysed later. If the Charlton data are excluded from the analysis the remainder still show a significant difference in mean IQ between the O and A₁ phenotypes ($t_{120} = 2.1$, 0.05 > $P > 0.01$).

Table 3 Frequencies of the ABO Phenotypes in Six of the Seven Villages

	n	A ₁	A ₂	O	B	A ₁ B	A ₂ B
Horton	112	0.45	0.03	0.45	0.06	—	0.01
Merton	83	0.35	0.02	0.45	0.11	0.05	0.01
Beckley	100	0.38	0.04	0.46	0.11	0.01	—
Wendlebury	130	0.43	0.08	0.42	0.05	0.01	0.01
Weston	96	0.43	0.03	0.39	0.08	0.04	0.02
Charlton	127	0.45	0.02	0.46	0.07	0.01	—

Although there are significant differences in mean IQ between the seven villages there is no evidence for spatial heterogeneity in the frequencies of the ABO phenotypes (Table 3). The sample can, however, be divided into those who were born in the Otmoor region and those who were born elsewhere and thus compare the frequencies of the ABO phenotypes and their associations with IQ among these geographically migrant and non-migrant groups. For the males, but not for the females, there are significantly fewer O phenotypes among the locally born than there are among those born outside the Otmoor region (Table 4). The males and females not born locally also have significantly higher mean IQs than those who were born locally, regardless of their blood group phenotype. In both the non-local females and the local males the mean IQ of the O phenotypes is higher than the mean IQ of the A₁ phenotypes, but the difference is not significant (Table 5).

Table 4 ABO Phenotypes Among the Locally and Non-locally Born

		A ₁	O	Others	Estimated gene frequency O
Females	local	32	32	5	0.68
	non-local	97	105	36	0.66
$\chi^2_2 = 2.98$, $P > 0.2$					
		A ₁	O	Others	Estimated gene frequency O
Males	local	56	30	13	0.55
	non-local	81	111	30	0.69
$\chi^2_2 = 12.5$, 0.01 > $P > 0.001$					

There is thus genetic evidence for at least two population groups in the area, but this alone cannot be responsible for the association between ABO blood group and IQ which the analysis reveals. Unfortunately we do not know how typical either the migrants or non-migrants are of the genetic populations they represent. Selective migration could account for the pattern of differences between the sexes and between the locally and non-locally born but only by accepting a direct or indirect association between the ABO blood groups and IQ.

Table 5 Mean IQs of A₁ and O Phenotypes among Locally and Non-locally Born

	n	Females A ₁	n	O	n	Males A ₁	n	O
Locally born	32	99.31 (2.4)*	26	99.96 (2.2)	51	100.53 (2.5)	25	103.44 (1.8)
Non-locally born	97	107.48 (1.2)	106	110.18 (1.1)	83	113.3 (1.6)	114	112.96 (1.1)

* Standard errors given in parentheses.

It is important to stress that our finding of this association between IQ and ABO blood groups in the Otmoor population does not necessarily imply that a similar association will exist in any other population. This will depend on whether the phenomenon is due to linkage disequilibrium or pleiotropy, and true pleiotropies of blood group genes are impossible to identify with the relatively crude characterisation of blood group specificities presently available.

The proportion of the genetic variance for IQ in this sample which is accounted for by this association is small, but it is not to be expected that a single marker locus will be associated with a large proportion of the variance. Thoday⁸ strongly advocated this approach to the analysis of continuous variables in human populations, especially psychometric variables, and our results suggest that the approach is worthwhile.

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Collateral Innervation of Muscle Fibres by Motor Axons of Dystrophic Motor Units

MUSCULAR dystrophy refers to a group of genetic degenerative diseases in which the skeletal muscle fibres undergo a progressive disintegration and are eventually replaced by fat or connective tissue. Besides these degenerative changes resulting in severe atrophy and paresis, the histological study of biopsy specimens also discloses scattered small areas of basophilic sarcoplasm containing increased RNA and nuclei with prominent nucleoli¹⁻³. The true incidence and functional significance of such signs of attempted muscle fibre regeneration are still uncertain. The electrophysiological data to be presented emphasise that motor axons innervating motor units affected by the disease retain the capacity to successfully innervate additional muscle fibres through collateral sprouts.

Detailed electromyographical studies were performed on seven male patients, aged 7 to 15 yr, with typical X-linked (Duchenne) muscular dystrophy. Standard concentric needle electrodes were used to pick up the potentials of single motor units (the muscle fibres innervated by a single motor axon) during very slight voluntary contraction. The potentials were recorded on magnetic tape and, when played back for detailed analysis, they were made to trigger the cathode ray oscilloscope time base. A delay line in the amplifier input allowed the initial 3 ms before the tripper point on the main potential deflection to be displayed on the oscilloscope screen⁴. Several potentials of the same motor unit can thus be either superimposed on a still photographic film (Fig. 1A-C) or recorded one below the other on a slowly moving film (Fig. 1D). A characteristic motor unit potential recorded in the deltoid of a normal adult volunteer (Fig. 1A) has about 300 μ V peak voltage and its total duration does not exceed about 10.5 ms. In patients with spinal muscular atrophy, many motor axons degenerate and the corresponding muscle fibres undergo neurogenic atrophy; the healthy motor axons present an extensive sprouting of collaterals which reinnervate the neighbouring denervated muscle fibres^{5,6}. In a muscle with such partial neurogenic atrophy the motor unit potentials are of course fewer in number but they present an increased voltage and sometimes also an increased duration^{7,8}. By analysing these motor unit potentials with the method described above, we recently demonstrated another characteristic sign of collateral reinnervation, namely the occurrence of many late components (Fig. 1B) which are delayed by up to about 50 ms with respect to the initial main deflection⁴.

In the affected muscles of patients with X-linked muscular dystrophy, the motor unit potentials are typically reduced both in voltage and duration⁷⁻⁹. We were surprised to find that quite a few of the dystrophic motor unit potentials also present late components (Fig. 1C) because it has generally been held that collateral reinnervation is a feature of neurogenic, but not of myogenic, atrophies. The late components recorded in dystrophic muscles are not an artefact resulting from repeti-

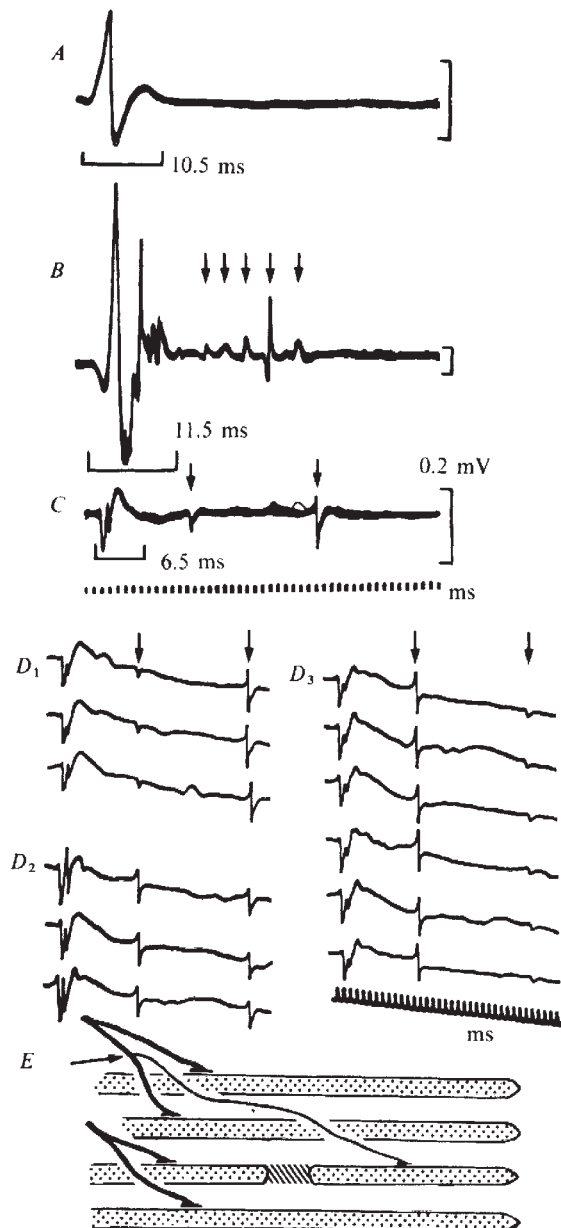


Fig. 1 Motor unit potentials recorded from the deltoid muscle with a concentric needle electrode (Disa model 13K0511) in, *A*, a normal 35-yr-old male subject; *B*, in a female patient 12 yr old with spinal muscular atrophy (Wohlfart-Kugelberg-Welander syndrome, juvenile type) since the age of 3; and, *C*, *D*, in another male patient 8 yr old with X-linked (Duchenne) muscular dystrophy since the age of 1. The potentials trigger the oscilloscope time-base and are recorded with an ADYU delay line of 3 ms. About seven sweeps are photographically superimposed in *A-C*. Arrows point to the late components. In *D*, the motor unit potential of *C* is displayed on moving film. Notice the changes in wave form when the recording needle is slightly rotated between *D*₁-*D*₂ and *D*₂-*D*₃. *E*, Tentative diagram showing how a muscle fibre segment isolated by focal necrosis could be innervated by a collateral sprout of a preterminal motor axon (arrow).

tive discharges. They represent muscle fibre activities which are triggered by the action potential of the same motor axon with a longer, but consistent, delay. For example, when the recording concentric needle electrode is slightly rotated in the muscle (Fig. 1 *D*₁-*D*₂ and *D*₂-*D*₃), the size and wave form of the several components of this motor unit can be made to vary independently while their respective latencies remain stable. As expected, the changes in wave form are more important for the two small late components than for the main

motor unit potential when the position of the electrode tip changes slightly with respect to the corresponding muscle fibre(s).

Extensive studies on the proximal and distal muscles of patients at different stages of the disease show that the phenomenon is of rare occurrence or absent for the motor unit potentials of normal configuration in the muscles at a pre-clinical stage, and also for the severely disintegrated motor unit potentials in the muscles with severe atrophy and fibrosis in advanced cases. By contrast, in the moderately affected muscles, many genuine late components are recorded in motor unit potentials which present already definite myopathic changes (polyphasia, reduced voltage and duration).

The motor axon innervating such dystrophic motor units thus retains the capability to produce collateral sprouts forming functional neuromuscular junctions on neighbouring muscle fibres. This observation does not seem to agree with the recent proposal that human dystrophies could result from a primary lesion of the motoneurons¹⁰. This issue is important and we have been careful to limit the present paper to evidence consistently recorded in human patients with genuine X-linked dystrophy, as shown by the clinical features, the marked elevation of serum creatine phosphokinase and the unequivocal electromyographic signs of myopathy in the proximal muscles. It should be pointed out that the evidence for denervation and neurogenic atrophy in Duchenne dystrophy¹¹ is still rather controversial, for it was obtained in a foot muscle (extensor digitorum brevis) which discloses neurogenic changes even in normal young subjects with no neurological disease¹².

Our results raise the question of which muscle fibres become available for accepting the collateral innervation in a dystrophic muscle. One possibility is that muscle fibres would become denervated but remain excitable in these muscles. This possibility should not be excluded but it seems to us rather unlikely; it implies that, in human X-linked dystrophy, the process of progressive disintegration should paradoxically involve the motor axon before the corresponding muscle fibres if the latter were indeed to be made available for accepting innervation by a new axon sprout. The well documented dropping out of muscle fibres from motor unit potentials as the dystrophy progresses indicates rather that the motor axon remains functional until only a few muscle fibres remain excitable in the motor unit. The current interpretation of this fact emphasises that the process of progressive disintegration must involve the muscle fibres before the corresponding motor axon⁷⁻⁹. This concept remains valid even if the primary dystrophy defect proves to be the lack of a trophic factor inadequately supplied by the motoneurone to its axon, which is our preferred form of the neurogenic hypothesis.

If no significant numbers of viable muscle fibres lost their innervation to become able to accept the collateral sprouts, the latter can only innervate newly formed muscle fibres. This raises interesting possibilities because muscle regeneration processes are known to occur in dystrophy¹⁻³. Our suggestion (Fig. 1E) is based on the observation that the dystrophic degeneration sometimes only affects part of the long muscle fibre and that the fibre segment thus disconnected from the endplate zone by the focal necrosis will reform its membrane and even display histological evidence of regenerative activity^{1,13}. Such viable isolated segments thus become new muscle fibres which can be innervated by an axon collateral. The delay of their action potentials with respect to the main motor unit spike would be related, among other things, to the longer conduction distance and the slower velocity in the thin axon collaterals (Fig. 1E, arrow). As well as regeneration of muscle fibre segments isolated by focal necrosis, one must consider both longitudinal splitting of muscle fibres into several daughter fibres and the formation of new fibres from myotubes and satellite cells¹⁴. The relative importance of these various regenerative processes for supplying viable muscle fibres in dystrophic muscles remains to be evaluated.

In any case, as newly formed muscle fibres are known to be

present in dystrophic muscles³ and as genuine processes of collateral innervation have now been disclosed (Fig. 1C and D) it becomes difficult to escape the suggestion that new muscle fibres formed by segmentation, longitudinal splitting and (or) regeneration must become enrolled into existing motor units and activated in voluntary contractions. The contribution of collaterally innervated fibres to the mechanical force output of the diseased muscle should be assessed and the nature of the muscle fibres concerned should be examined in further studies. The reparative process of collateral innervation described in this paper is considered likely to delay the onset and progression of the clinical weakness in patients with X-linked muscular dystrophy.

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Preliminary Characterisation of the Caudal Organ Secretion of *Apodemus flavicollis*

THE existence of a secretory organ lying along the proximal third of the undersurface of the tail of the mouse *Apodemus flavicollis* has been known for some time¹. Such organs are present in other species of the same genus^{2,3}. The organ is composed of holocrine sebaceous tissue up to 1 mm deep and produces a thick milky exudant which can sometimes be expressed by gentle lateral pressure on the tail. The organ is anatomically similar to that found in the tails of *Perognathus* and *Chaetodipus*⁴. The function of the organ in *A. flavicollis* is not known¹, but it is possibly involved in olfactory recognition of territory, or self advertisement. It may also be used in mate and nest marking. Its secretion has a characteristic odour to the human nose resembling the odour of voles rather than the odour of laboratory mice. Microscopically, the organ is plainly visible in adult male mice, but much less so, if at all, in both adult females and juveniles of either sex.

A population of *A. flavicollis* inhabiting a *Quercus-Carpinus* forest close to Brno, Czechoslovakia, was studied by

break-back trapping during the summer of 1972. Trapped animals were weighed and their sexual and reproductive conditions determined. Females were recorded as having the vagina perforate (in breeding condition), imperforate (not in breeding condition unless copulation plug present), pregnant and/or lactating. Males were recorded as having testes scrotal (in breeding condition) or abdominal (not in breeding condition). The mean length of the testes was recorded to provide an indication of sexual maturity. These data, from twenty-one specimens, were supplemented by data from a further five specimens caught in Sussex in the autumn of 1972. From each trapped animal the proximal 12 to 15 mm of the skin of the tail was sealed in a small tube with one drop of ultra pure xylol, which acted both as a solvent for the secretion and as a preservative for the tissues.

Before analysis, the tail organ was ground in the tube. Extra xylol was added only if the seal of the tube had allowed some evaporation to occur. The extracts were analysed by gas-liquid chromatography (see legend to Fig. 1).

The tail gland secretion was found to consist of a mixture of twenty-six principal components or component complexes with boiling points ranging from 210° C to 300° C (Fig. 1A). Only one of these components, peak *i*, is present in any amount in the skin of the distal portion of the tail (Fig. 1B). This peak has also been found in chromatograms from skin extracts from the head and neck region of both *A. flavicollis* and the related species, *A. sylvaticus*.

The chemistry of tail organ secretion is the subject of a continuing study and will be reported elsewhere but it is known that peaks *a* to *m* form homologous series of long chain esters of long chain alcohols, with molecular weights of from 396 to 466 (D. M. S., unpublished observation). What is reported here is the amount of difference found in tail organ secretions from different sex and age classes of the population. There is some variation within each age and sex class but this is much less than that between classes. The examples chosen for illustration are completely typical of their represented classes. Adult males are characterised by having many peaks, especially many of high boiling point (Fig. 1A). Adult females have, typically, no peaks of high boiling point and only a small development of the components with low boiling point (Fig. 1C). Female tail secretion is remarkably similar to that of juvenile males (Fig. 1D) and to juvenile females. In essence, the proximal portion of adult female, juvenile female and juvenile male tail is chemically similar to distal tail skin or general skin of adult males.

At what stage in development does the tail organ of the male produce typical male substance? An indication of this may be gained from the state of sexual maturity. The average testis length of adult males in the Brno population in 1972 was 13.85 mm and the average body weight 28.24 g. In every respect (that is, coat colour, tail length and hind foot length) the animal represented in Fig. 1E resembled an adult more than a juvenile. Its chromatogram shows a stronger relative development of components *k*, *l*, *o*, *p*, *r* and *x* than is apparent in Fig. 1D. In the development of components *k*, *l*, *o*, *v*, *x* and *z*, however, it resembles more an adult male (Fig. 1) than it does an adult female (Fig. 1C).

A further point of interest arises from these observations. A cluster of peaks of boiling point between 150° C and 180° C was observed in all samples analysed. By far the greatest development of these occurs in juvenile mice of both sexes. The same components also occur in adult males but, relative to the rest of the peak pattern, they are in very much smaller amounts. They are practically, but not completely, absent from secretions produced by pregnant and lactating mice. There is some evidence that they occur in perforate (that is, sexually mature) females that are neither pregnant nor lactating.

These observations seem to suggest that the function of the secretion is sociological rather than physiological. By producing a secretion similar to that produced by their mothers, juvenile males are possibly treated as females by adult males

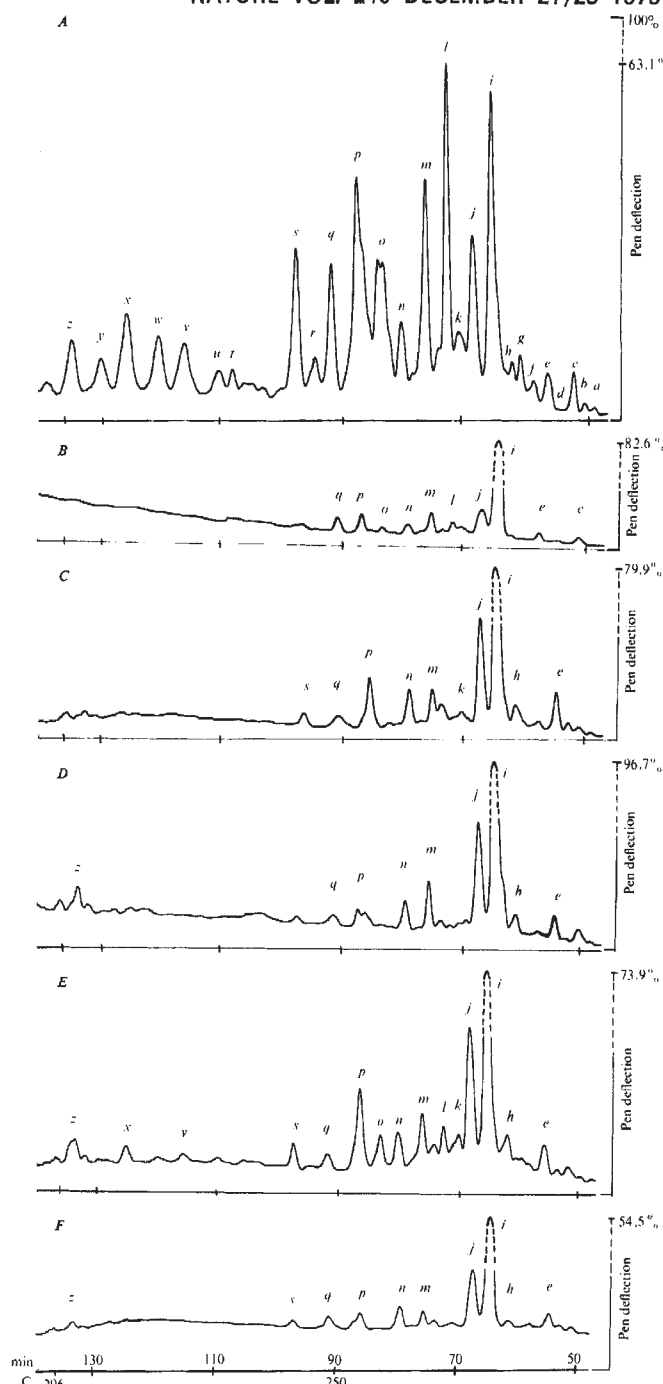


Fig. 1 A, Chromatogram of caudal organ extract of adult male *Apodemus flavicollis* (weight of mouse, 36.8 g; testes scrotal; mean testis length, 14.5 mm). Letters *a* to *z* indicate twenty-six principal components or complexes of components. Sample size 1.5 μ l. Pye Unicam gas-liquid chromatograph series 104, dual heated flame ionisation detectors. Columns: 1.5 m $\times$ 4 mm glass 3% dimethyl silicone gum on 100-120 mesh Diatomite CQ. Carrier; N_2 at 54 ml min⁻¹. Column temperature: 170° C for 5 minutes then 1° C min⁻¹, 296 to ∞ . Detector temperature: 300° C. Injection temperature: 235° C. B, Chromatogram of skin of adult male *A. flavicollis* from the non-glandular (that is, distal) region (weight of mouse, about 35 g; testes scrotal; mean testis length, 14.2 mm). Sample size 2 μ l. (Chromatography details as in A.) C, Chromatogram of caudal organ extract of adult female *A. flavicollis* (weight of mouse: 33.6 g, pregnant, five embryos). Sample size 2 μ l. (Chromatography details as in A.) D, Chromatogram of caudal organ extract of juvenile male *A. flavicollis* (weight of mouse, 16.6 g; testes abdominal; mean testis length, 6.2 mm). Sample size 3 μ l. (Chromatography details as in A.) E, Chromatogram of caudal organ extract of juvenile male *A. flavicollis* (weight of mouse, 19.0 g; testes abdominal/scrotal (?); mean testis length, 8.9 mm). Sample size 1.5 μ l. (Chromatography details as in A.) F, Chromatogram of caudal organ extract of adult female *A. flavicollis* (weight of mouse, 18.1 g; perforate, non-parous). Sample size 3 μ l. (Chromatography details as in A.)

and are not subject to attack. In addition, they produce a juvenile component complex which, probably by virtue of its relative abundance in this age class, may assist in identifying them as juveniles. As soon as they are physiologically capable of competing with the males, they produce a secretion more like that of their father than their mother, although the low-boiling point fraction is still present. If a similar situation exists in other species of rodents, and there is some evidence to suggest it does (D. M. S., unpublished data), then it could help to explain why juvenile male rodents are not attacked by adult males^{5,6}. Further, if the quality of secretion is capable of change in response to hormonal changes resulting from increasing population pressure or onset of breeding, the differential tolerance of males to one another during different phases of a population cycle could be explained^{7,8}. Questions arising from these suggestions are the subject of continuing studies.

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Distribution and Immunology of Mammalian Nerve Growth Factor

We report here a series of experiments in which we have measured the amount of nerve growth factor (NGF) present in the serum of a number of species. We have also compared the immunological properties of the serum NGF with purified NGF from mouse salivary gland and from a snake venom.

'Nerve growth factor' describes that group of proteins which, when injected into neonate animals, produces hypertrophy and hyperplasia of the neurones of the sympathetic nervous system (for leading references, see ref. 1). It also produces characteristic outgrowths of fibres from explanted embryonic chick sensory or sympathetic ganglia; this response forms the basis of an *in vitro* assay method developed by Levi-Montalcini *et al.*². We used a modification³ of this assay. Levi-Montalcini and her coworkers have reported that NGF is a normal component of the sera of all mammals^{4,5}, including man, but the experimental details seem to refer only to mouse serum^{6,7}. In the present study, the direct assay of a number of different sera, either undiluted or at dilutions of up to a hundred-fold, gave essentially negative results. Suboptimal results were observed in some cultures but were below the standard⁴ required to define activity. To improve sensitivity, a fractionation procedure based on the known^{8,9} basic properties of NGF was then used. The sera were concentrated and desalted by pressure dialysis and lyophilised. Batches were reconstituted in a buffer solution containing 2-amino-2-methylpropane-1,3-diol/HCl (0.01 or 0.05 M, pH 8.5), dialysed against the buffer for 18 h, and then chromatographed on QAE A-25 Sephadex equilibrated in the same

conditions. Activity was detected typically in the unabsorbed fractions (~1–5% of the whole protein). The serum of the adult male mouse proved to be an exception: the active material was retained by the resin but could be eluted by application of a higher concentration of starting buffer (0.1 M). It is not clear whether this atypical behaviour reflects a fundamental difference in the nature of the protein from this source, or whether it is indicative of some interaction between the factor and other serum proteins. On the basis of the activities of the individual fractions, the specific activities of the different sera are given in Table 1. The results are the means from several experiments; occasional negative results were obtained, presumably because of the very low levels of NGF in the sera and of the variation from batch to batch. It can be seen that NGF was detected in all the sera examined, the level varying by approximately 200-fold.

Table 1 NGF Activity in Sera

Animal	NGF activity (biological units ml ⁻¹)
Mouse ♂	10–20
Mouse ♀	0.7
Human	1.5
Pig	0.2–0.4
Horse	0.1–0.5
Foetal calf	0.1–0.2
Cockerel	0.2

The level of NGF in male mouse serum deserves special comment. A specific activity of 10–20 biological units ml⁻¹ was obtained for this material, a level which should have been detected easily by direct assay of the whole serum after appropriate dilution. Our failure to do so may mean that whole serum contains an inhibitor which is removed on fractionation. The active fractions from the male mouse and from human serum were further fractionated by gel filtration on Sephadex G-100. In each case, active material was eluted in a fraction containing 5–15% of the applied sample and with an apparent molecular weight in the range 10,000–70,000. These fractions were used in the experiments with antisera described below.

Nerve growth factor can be isolated from mouse submaxillary gland in two forms: (1) a high molecular weight aggregate (7S NGF) (ref. 10) containing NGF and two other inactive proteins and (2) as an active homogeneous entity (β subunit, 2.5S NGF) containing two identical polypeptide chains^{11–13}. The β subunit has a molecular weight of about 30,000 and contains no carbohydrate⁸. Snake venoms also contain NGF, and that from *Vipera russelli* has been shown to be a glycoprotein containing 20% carbohydrate and with a molecular weight of about 37,000 (ref. 9). The non-carbohydrate part is about the same size as the dimeric β subunit and the two have comparable specific activities *in vitro* but the degree of structural homology, if any, is unknown.

Antisera to both forms of mouse salivary gland NGF (7S and β subunit) and to the NGF from the venom of *Vipera russelli* were raised in rabbits in conventional manner. Using successive two-fold dilutions, the highest dilution of antiserum required to block the activity *in vitro* of one biological unit⁴ of NGF was determined for all the possible combinations of antigen and antibody (Table 2). It can be seen that the two forms of NGF from mouse salivary gland are very similar antigenically but are quite different from the NGF from the venom of *Vipera russelli*. The biological activity of the mouse antigen is either unaffected or blocked only by very high concentrations of the antibody to the snake antigen and *vice versa*. This is not surprising as, although the proteins have similar biological effects, the degree of homology between the active polypeptide chains of the NGF from the two species

is unknown and, further, the antigen from the snake is a glycoprotein whereas that from the mouse is not. The carbohydrate components of glycoprotein antigens are known to be capable of contributing to or determining the immunological properties of the molecule¹⁴. What is surprising, however, is that the NGF present in the serum of mouse and of man is, in terms of the test described above, practically indistinguishable from the snake venom NGF and not at all like the NGF present in mouse salivary gland. The precise significance of these findings is not clear. The NGF in mouse serum detected by our procedure cannot be exactly the same as the NGF in the salivary gland and it is tempting to suggest that the serum factor may be a glycoprotein in the same way as the venom NGF. The similar immunological properties of the molecules could then arise from common carbohydrate determinants. A number of workers have suggested, however, that the NGF in mouse serum may be derived chiefly from the submaxillary gland^{7,15}. If the serum protein is actively synthesised in the gland, it must be modified on secretion such that its antigenic properties become altered. A modification of this type could be introduced if carbohydrate is attached to the newly synthesised protein to promote its transport into the extracellular environment. Such a general role for the carbohydrate moieties of glycoproteins has been suggested by Eylar¹⁶, to account for the observation that the majority of extracellular proteins, and especially serum proteins, contain carbohydrate. Consistently with other reports^{17,18}, however, we have failed to detect active material in the submaxillary glands of other mammalian species and the most reasonable interpretation of the present results is that the NGF detected in the serum of all the species tested (including mouse) does not arise from the submaxillary gland but is synthesised elsewhere.

Table 2 Immunological Properties of NGF

Antigen	Antibody titres—reciprocal dilution blocking the activity of 1 BU		
	MSG 7S	MSG β subunit	<i>Vipera russelli</i>
MSG 7S	3,000–6,000	6,000	0–15
MSG β subunit	6,000	6,000	≤ 3
<i>Vipera russelli</i>	≤ 3	≤ 3	1,500–3,000
Human serum	0–6	15	1,500
Mouse serum	0–6	≤ 3	1,500–3,000

The value obtained in the present experiments for the NGF content of the serum of the male mouse (10–20 units ml⁻¹) should be compared with the values of 15 units ml⁻¹ and 180 units ml⁻¹ reported by Hendry¹⁵ and by Johnson *et al.*¹⁹, using radio-immunological methods based on mouse salivary gland NGF. That any measure of agreement should exist at all demands some comment. The radio-immunoassay procedure requires that the NGF in the serum should be recognised and bound to the antiserum prepared against the NGF from mouse salivary gland, whereas the present findings show that the biological activity of serum NGF is hardly affected by this particular antiserum. It is, of course, true that combination of antigen and antibody does not necessarily lead to loss of the antigen's specific biological activity^{20,21}. Whether this applies in the present case can, however, only be settled by careful immunological and biological testing on the same samples.

It is also possible that mouse serum contains more than one form of NGF and that in our procedure only one form is isolated. This is conceivable as it has been suggested recently that NGF is not produced in any specific organ in the animal but is synthesised in a variety of different tissues⁵. The proteins produced in this way may not have precisely the same immunological properties. In

any event, the findings suggest that the biological significance of the form of NGF present in mouse submaxillary gland is open to question.

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Electrophysiological Evidence for Existence of Neurones sensitive to Direction of Depth Movement

RECENT psychophysical evidence suggests that there are movement detectors in the human visual pathway selectively sensitive to different directions of motion in three-dimensional space¹⁻⁵. In animals (such as the cat) there are single nerve cells with properties which suggest that they signal when an object is moving in depth away from the plane of binocular fixation⁶.

Regan and Spekreijse's^{7,8} report of electrical signals generated by the human brain and specific to changes in retinal

disparity (that is, to stimuli which appeared to move in depth) suggested the possibility of establishing a stepping-stone between subjective studies of human depth perception and the properties of disparity-sensitive single neurones in animal brains. We have recorded electrical brain responses in man, and report here evidence for the existence of neurones that respond differently to stimuli that move away from and towards the plane of binocular fixation.

The left and right eyes viewed a 5° pattern of randomly-arranged black dots that differed in only one respect. This was that the central 2° of the left eye's pattern could be moved from side to side. When the left eye alone was used, the subject saw the central 2° move from side to side (monocular condition). When the two patterns were viewed by both eyes in binocular fusion, however, the movement produced a compelling illusion that the central 2° of the pattern was moving backwards and forwards in depth (stereoscopic condition)⁸⁻¹¹. (The stimulus did not really move in depth at any time. All depth changes were illusions produced by changing retinal disparity. For brevity, however, the phrases 'depth changes' and 'position in depth' will be used in referring to these illusions.) The initial displacement in depth of the 2° target could be set so that it appeared to be located in space either in front of or behind the plane of binocular fixation. Ocular convergence was monitored by means of nonious lines. These enabled a change in angular convergence of 0.5 arc min to be detected (much smaller than the disparity change required to elicit an appreciable evoked potential (EP)).

In some experiments a bar stimulus was used. The left and right eyes viewed identical patterns of randomly-arranged 2 mm dots. These patterns defined the fixation plane. A black bar that subtended $2^\circ \times 8'$ was located 1° to the right of the central nonious lines. The left eye's retinal image of this bar could be moved from side to side.

Chlorided silver disks were attached to the occipital scalp (1 cm above the inion) and to the left mastoid. Electrical brain responses (EPs) were recorded between these electrodes by means of an averaging computer⁸. The right mastoid was grounded. The bandpass of the recording system was 0.08 Hz to 40 Hz (3 dB down). Reducing the high frequency cut from 40 Hz to 12 Hz did not, however, appreciably change our amplitude measures but did reduce noise, so this bandpass was used to record the waveforms of Fig. 1.

A prominent, positive-going wave followed by a negative-going wave was generated for each movement of the stimulus. For both directions of stimulus movement and for both stereo and mono conditions, the latency to the peak of the positive-going wave was roughly 120 ms and the latency to the peak of the negative-going wave was roughly 220 ms.

These responses are similar to those observed by Regan and Spekreijse except that they did not observe such large negative-going waves⁷. When we used Julesz patterns similar to those used by Regan and Spekreijse, however, the negative-going wave was much less prominent.

Figure 1A shows that stereoscopic (stereo) stimulation gave quite different responses to those elicited by monocular (mono) stimulation. The upper two records in Fig. 1A show the brain's electrical responses to a target that appeared to move abruptly backwards and forwards in depth at a rate of 0.9 Hz (stereo condition). The target remained in front of the fixation point in one recording and behind the fixation point in the other recording. It is clear that for a target located in front of the fixation plane, quite different response amplitudes were recorded for movements directed towards and away from the eye. For stimuli located behind the fixation point the two directions of movement also gave different responses; in this case, however, response amplitudes were roughly similar and the difference was one of waveform. The two lower traces of Fig. 1A show that these directional asymmetries disappeared when the retinal image movements were not accompanied by changes in retinal disparity (mono

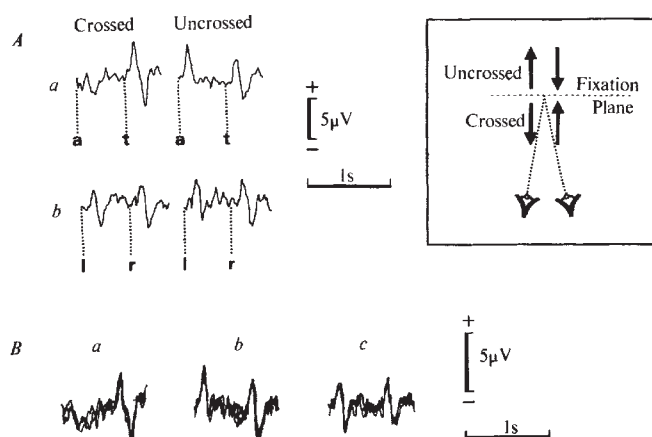


Fig. 1 A, Electrical brain responses (a) to stereo movements for targets located in front of and behind the plane of binocular fixation and (b) responses to monocular control stimuli similarly located in front of and behind the fixation plane. Insert illustrates the apparent positions and directions of movement in space in the two stereo conditions. B, Variability illustrated by three superposed traces for, a, stereo crossed; b, stereo uncrossed; and c, mono stimulation. In the stereo traces t means movement towards the eyes and a means movement away from the eyes. In the mono traces l and r refer to leftward and rightward movements respectively. Each trace is the average of 500 sweeps in A and 100 sweeps in B. The stimulus frequency was 0.9 Hz and the sweep duration 1 s. The stimulus target was a 2° circle of random black dots surrounded by an annulus of similar dots extending to 5° . The dots occupied some 1% of the total area. Mean stimulus luminance 110 cd m^{-2} . Distance from eye to target 75 cm. The stimulus displacement of $10'$ was the same in each condition.

condition). This finding also held when, in the mono condition, the right eye viewed an uncorrelated random dot pattern. It is important to note that: (a) in both stereo and mono conditions the left eye only received movement stimulation, and (b) these movements were identical in the mono and stereo conditions. In the stereo situation the right eye viewed a similar but static pattern, so that the brain could compute changes in retinal disparity (and thus the target appeared to move in depth), whereas in the mono situation the right eye was occluded so that the brain could compute only changes in retinal image position (so that sideways movements only were seen).

Figure 2 illustrates a second distinction between stereo and mono responses, and shows the effect of changing movement amplitude in the four stereo (a-d) and two mono control (e, f) situations. In the mono situation, response amplitude showed no further increase when stimulus amplitude was progressively increased beyond 5 arc min. In contrast, responses to stereo stimulation either grew progressively as stimulus amplitude increased, or did not saturate until stimulus amplitude exceeded 15 arc min. Thus, the relationships between response amplitude and stimulus excursion were quite different in the mono and stereo situations.

These results (Figs 1 and 2) held for each of a total of fifteen separate experiments.

A further point is that the data of Fig. 1A cannot be explained simply by assuming that different responses are elicited by movements in depth directed towards and away from the eyes. Figure 2 provides additional evidence that this explanation is insufficient. Independently of whether the target was in front of or behind the fixation point, movements directed away from the fixation point gave concave downwards curves (Fig. 2a, d) whereas movements directed towards the fixation point gave concave upwards curves (Fig. 2b, c). It is worth noting that from this it follows that differences in absolute amplitudes between the various types of stereo and mono responses must be considered in relation to stimulus amplitude (compare with Fig. 1).

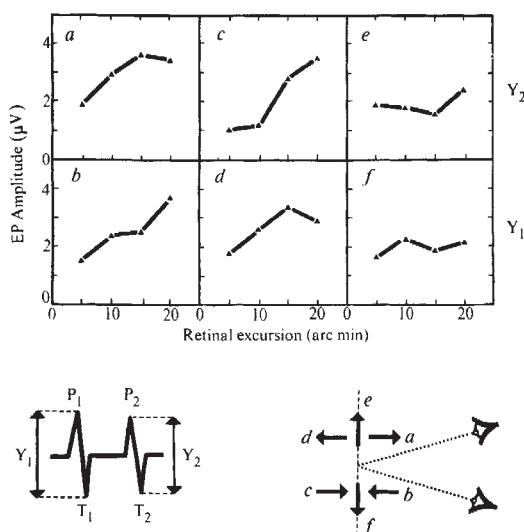


Fig. 2 Effect of stimulus amplitude on the electrical response of the brain. Each curve is a plot of response amplitude in microvolts (μ V) against the excursion of the left eye's target in arc min. *a, b*, Stereo crossed; *c, d*, stereo uncrossed; *e, f*, monocular stimulation. One insert shows how response amplitude was measured for targets moving towards the eyes (Y_2) and away from the eyes (Y_1) (see ref. 8, page 229, for a discussion of this definition of response amplitude). The other insert illustrates the target position and direction of movement for each of the four Stereo and two mono plots. The measurements were derived from averages of 100 sweeps, each of 1 s duration. The stimulus was a vertical $2^\circ \times 8'$ black bar located 1° to the right of the nonius lines. The fixation plane was defined by a 5° square pattern of random black dots that occupied roughly 1% of the area of the 110 cd m^{-2} background. Distance from eye to target was 75 cm.

Our conclusions hold for both bar and dot patterns, though there were some waveform differences. Although bar patterns gave smaller responses than dot patterns, the differences between stereo and mono conditions seemed clearer for bar patterns.

The findings of Figs 1 and 2 can be explained on the basis of the two following assumptions: (a) The way in which the brain handles information that a target's retinal disparity has changed depends on whether the target's mean disparity is crossed (that is, target in front of the fixation point) or uncrossed (target behind the fixation point). (b) Information of a change in retinal disparity is handled differently when the target is moving away from the fixation point than when it is moving towards the fixation point.

Assumption (a) would agree with psychophysical evidence for the existence of the disparity pools postulated by Richards¹²⁻¹⁵. Assumption (b) would agree with reports of psychophysical adaptation to the direction of movement in three-dimensional space¹⁻⁴. The distinction reported here between human brain responses to depth movements directed away from and towards the fixation point may reflect the activities of neurones selectively responsive to the movement of a target whose disparity is changing. Though they seem rare, such neurones have been found in cat by Pettigrew⁸ and in monkey by Zeki (personal communication). Further, the neurones reported by Pettigrew were selectively sensitive to disparity changes relative to the point of fixation (directed away from the point) which would agree with our data of Fig. 2.

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Experimental Creation of Unusual Neuronal Properties in Visual Cortex of Kitten

A NORMAL cat faces the visual world with an arsenal of feature-detecting neurones in its visual cortex. Virtually every cell is selectively sensitive to the orientation of an edge moving across its receptive field¹ and the whole gamut of possible orientations is represented among the complete population of neurones.

Undoubtedly visual experience itself, early in the kitten's life, plays at least some part in setting up these orientation-detecting neurones. The visually inexperienced kitten's cortex has large numbers of cells that are non-oriented (responding weakly to edges of any orientation) and even some that are totally unresponsive to visual stimuli^{2,3}. Indeed, kittens reared in restricted visual environments, consisting of edges of one orientation, fail to develop cortical cells responding optimally to contours of the orthogonal orientation^{4,5}, even if the exposure is remarkably brief⁶.

During a series of experiments to determine the exact kind of visual stimulation needed to produce the full complement of normal orientation-selective cortical neurones, we reared two kittens in such a manner that almost their entire visual experience was restricted to an array of coarsely-spaced white spots on a dark background. Our rearing procedure was very similar to that of Blakemore and Cooper⁵. Each kitten was housed in a darkroom from the time of eye opening and exposed to a spot pattern for about 2 h each day. The kitten was placed in a transparent cylindrical chamber suspended in the centre of a larger cylinder, on the inside walls of which the spots were displayed. With the kitten looking straight ahead the maximum and minimum viewing distances were restricted to about 8 cm and 32 cm respectively. If it looked far up or down the walls of the display cylinder the maximum viewing distance increased to about 55 cm.

For Kitten 1 the white spots were 0.5 cm in diameter, subtending a visual angle of about 1° to 3.5° for straight ahead viewing. The spots were irregularly scattered over the dark background with a minimum spot separation (centre-to-centre) of 4 cm (7° to 23°) and an average separation of about 7 cm (12° to 41°). For Kitten 2 the spots were 1.3 cm (2.5° to 9°) in diameter with roughly the same spacing. Kitten 1 had 80 h of this visual experience from 3-10 weeks of age, Kitten 2 had 52 h from 3-8 weeks.

Immediately after the period of restricted rearing, we used conventional methods to record activity from single neurones in the visual cortex. The kittens were paralysed with Flaxedil ($7.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) and anaesthetised with 75% nitrous oxide. The responses of the neurones were studied by back-projecting spots, bars, edges and so forth, on a tangent screen 57 cm in front of the eyes.

Histological reconstruction showed that the penetrations were made in area 17, in the area centralis projection area. The microelectrode was purposely driven down the medial

bank of the postlateral gyrus to ensure that it sampled from a wide region of cortex, crossing the borders of many cortical columns⁴. In both cases the electrode penetration was more than 3 mm in depth. As in previous experiments on kittens reared in restricted environments^{5,6}, we found no large regions of silent cortex that might have indicated massive degenerative changes. All receptive fields were within 10° of the area centralis and most were within 5°. There were no obvious differences between the two animals in average receptive field size, which was comparable to that for similar samples from adult cats.

Using line stimuli, we were able to classify all neurones into one of the five categories found in normal and visually inexperienced kittens. (1) Orientation-selective: having a clear preferred orientation for moving bars, with no response at all to a bar of the orthogonal orientation. (2) Orientational bias: having an obvious preference for one orientation, but responding somewhat to all. (3) Non-oriented: firing equally for all orientations. (4) Pure direction-selective: firing to any stimulus moving in a preferred direction but with no response in the reverse direction. (5) Visually unresponsive: with spontaneous activity but no response to our repertoire of visual stimuli.

One feature of these cortical neurones was, however, most unusual. A large proportion of the visually responsive cells discharged just as vigorously, if not more so, to moving spots of roughly the angular size experienced during rearing as to extended contours. Thus many non-oriented cells had a preference for spots (rather than long lines) moving in any direction: Fig. 1*a* shows responses from such a cell (recorded from Kitten 1), which preferred a spot of about 2° in diameter. The pure direction-selective cells also responded vigorously for spots moving in the preferred direction (Fig. 1*b*) and most cells, classified as orientation-selective or orientational bias on the basis of their responses to long lines, responded at least as well to a small spot moving along the correct axis as to an optimally oriented line (Fig. 1*c*).

The results are summarised in Table 1, which shows the total number of units of each type and the number of cells responding at least as well to spots of the appropriate size as to long lines. A large proportion of the visually responsive cells (88% in Kitten 1, 89% in Kitten 2) had this unusual property.

These results cannot be attributed to the young age of the kittens, since a control animal (Kitten 3), reared in a normally illuminated laboratory environment and recorded from only 5.5 weeks of age, had an essentially adult visual cortex (Table 1). Almost every cell was clearly orientation-selective and only 16% responded equally well to 1°–5° diameter spots as to elongated bars. Nor can the observations be explained by the period of dark rearing until 3 weeks the unusual regime of visual stimulation: in a second control animal (Kitten 4), which was treated identically but reared in an environment of vertical stripes⁵ for a total of 49 h from 3–7 weeks, only

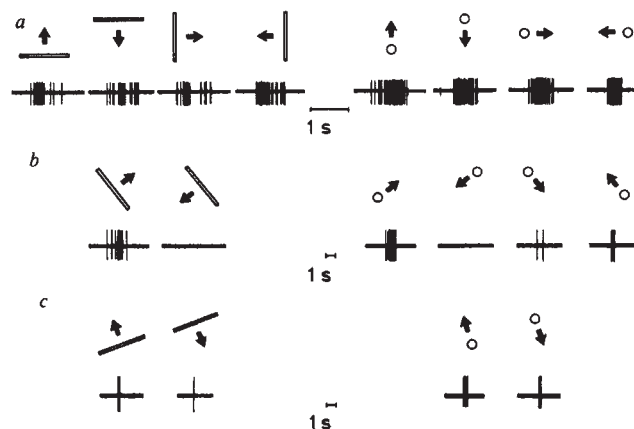


Fig. 1 Oscilloscope records of responses from three cortical neurones recorded in Kitten 1. The stimulus and its direction of movement across the receptive field are shown above each record: a long white bar (approximately $20^\circ \times 0.25^\circ$) was used for the responses shown on the left, and a 2° diameter white spot for those on the right. *a*, Unit K103-R14: a non-oriented cell. *b*, Unit K103-R38: a pure direction-selective cell. *c*, Unit K103-R12: an orientational bias cell, which preferred the orientation shown, but also gave some response to the orthogonal orientation.

13% of neurones responded equally or better to a small spot as to a bar (Table 1).

In the normal adult cat's cortex there is one class of orientation-selective neurone that prefers edges of a limited length and has little or no response to an extended border. These hypercomplex cells⁷, which are rather rare in area 17, usually therefore respond much better to a spot of a particular diameter than to a long edge. We tested all orientation-selective cells in the experimental kittens with lines of various lengths and not surprisingly most of the cells that actually discharged much more strongly for a spot than a bar also demonstrated length selectivity for bars, typical of hypercomplex cells. Many cells were, however, not of this extreme type: they simply responded just as well to a spot, much smaller than the total length of the receptive field, as to a bar that filled it. The characteristic is almost never seen in the normal adult cat's cortex.

In conclusion, early visual experience of extended straight edges seems to be a principal requirement for the establishment or maintenance of a totally normal visual cortex. Significantly, Kitten 2, exposed to large diameter spots with edges approximating to an extended contour, had many more orientation-selective cells (52% of all neurones) and fewer non-oriented units (14%) than Kitten 1 (23% and 33% respectively), which saw only small diameter spots and therefore very restricted contours. The two control animals (Kittens 3 and 4) both experienced extended contours in their environments and virtually all the neurones sampled from them were orientation selective (93% and 100% respectively). We shall consider

Table 1 Classification of Cortical Neurones Recorded from Two Kittens Exposed to Spots During Development, and from Two Control Animals

	Orientation-selective	Visually responsive neurones				Visually unresponsive	Overall total
		Orientational bias	Non-oriented	Pure direction-selective	Total		
Kitten 1 (small spots)	9 (6)	9 (8)	13 (13)	2 (2)	33 (29)	6	39
Kitten 2 (large spots)	22 (19)	6 (6)	6 (5)	2 (2)	36 (32)	6	42
Controls							
Kitten 3 (normal vision)	29 (3)	1 (1)	0	1 (1)	31 (5)	0	31
Kitten 4 (vertical stripes)	24 (3)	0	0	0	24 (3)	0	24

Numbers in parentheses refer to the number of cells in each category responding at least as well to spots of the appropriate size as to long lines.

this question of the relationship between the proportion of orientation-selective cells and the type of visual experience more fully in a future paper.

Prolonged exposure to edges of a particular curvature seems to create neurones that exhibit a preference for just such stimuli. Thus cells in the developing visual cortex seem to have a degree of 'adaptive' plasticity—an ability to match their properties to an unusual visual environment—that normally they would rarely be called on to employ.

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Optical Recording of Impulses in Individual Neurones of an Invertebrate Central Nervous System

FOR many purposes it would be a great advantage if one could measure the activity of individual neurones without the use of an electrode. Since the discovery of changes in the optical properties of axons that occur during the action potential^{1,2}, it has been possible, in principle, to accomplish this using optical techniques. Recently, this was achieved in a giant axon using a merocyanine dye; in a stained axon a single action potential gave rise to a fluorescence increase which was detectable with a signal-to-noise ratio greater than 10:1 (ref. 3). Because the membrane area observed in the giant axon experiments was about 10³ times larger than that of a 50 μ m cell body, it was by no means evident that this fluorescent probe would be sensitive enough to monitor electrical activity in smaller cells. Happily, it is; with the merocyanine dye, we are able to detect action potentials in individual sensory neurones of leech segmental ganglia. We hope that this technique can be developed into a powerful tool for studying the functional organisation of populations of neurones.

The leech segmental ganglion (see ref. 4, Fig. 2) seemed a favourable preparation because the ganglion is transparent and its cells are arrayed in a highly stereotyped manner in a monolayer cortex. In addition, many of the cells have been functionally identified. Ganglia from *Hirudo medicinalis* were incubated for 20 min in a Ringer solution^{3,4} which contained a merocyanine dye in a concentration of 0.05 mg ml⁻¹ together with 0.02% Pluronic F-127 (a non-ionic surfactant polyol). The dye mixture was then replaced by a leech Ringer⁴ containing an increased calcium concentration (5 mM instead of 1.8 mM). In this solution the resting and action potentials of leech neurones are maintained with enhanced stability. To reduce photodynamic damage^{5,6} to the cells, the bathing fluid was bubbled with nitrogen, but, despite this precaution, the photodynamic effect associated with this dye limited the duration of the experiments. One of the sensory neurones⁴ was

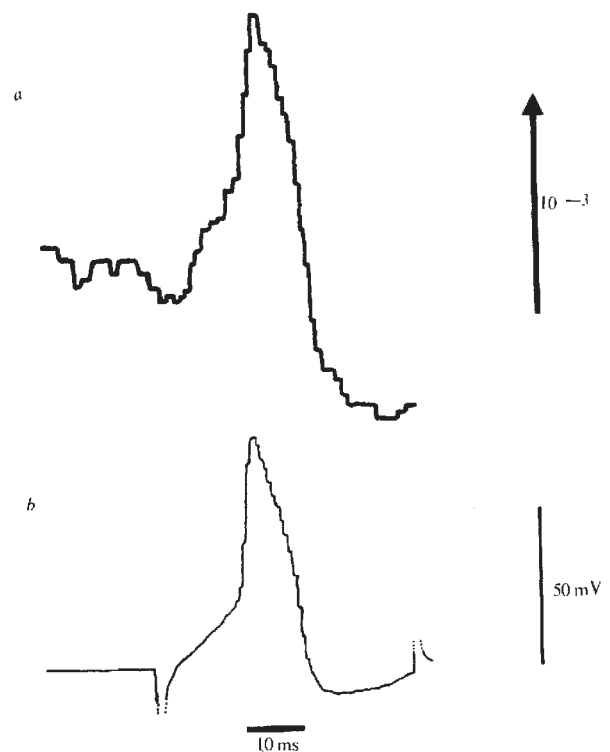


Fig. 1 Simultaneous measurement of fluorescence intensity (a) and membrane potential (b) in a nociceptive (N) cell of a leech segmental ganglion. In this single sweep there was an obvious fluorescence increase during the action potential. The vertical arrow to the right of the intensity trace in each figure represents the stated value of the change in intensity, ΔI , divided by the resting intensity, I_r , in a single sweep. The merocyanine dye was 5-[(3-sulphopropyl-2(3H)-benzoxazolylidene)-2-butenylidene]-1,3-dibutyl-2-thiobarbituric acid, and is available from Eastman Kodak Co. as 'merocyanine'. The response time constant of the light recording system was 2.2 ms. The signal averager used for recording sampled once every 0.5 ms and this accounts for the jagged character of the records. Cell body diameter, 68 μ m. Temperature, 21°C.

impaled with a microelectrode filled with 4 M potassium acetate (resistance 40 to 100 M Ω) and connected to an electrometer (WPI, M-4) which permitted simultaneous stimulation and potential measurement. Light from a Hanovia xenon arc lamp (448C1 or 901C11) was collimated, passed through an interference filter (540 ± 15 nm), and then focused on the ganglion by means of the brightfield condenser on a Leitz Ortholux II microscope. The light collected by a $\times 20$ (numerical aperture 0.4) long-working-distance objective was passed through a Schott OG 590 filter, which attenuated the incident light (by 10⁴) and transmitted the emitted light. A pinhole 1.9 mm in diameter was placed in the objective image plane so that the input to the photodetector (EG&G, PV100A) was restricted to light emanating from a region of the ganglion 95 μ m in diameter. This pinhole was mechanically positioned over the image of the stimulated cell. The amplified outputs of the photodetector and microelectrode electrometer were fed into separate inputs of a signal averager for storage or averaging.

Figure 1 illustrates the simultaneous measurement of fluorescence (a) and potential (b), recorded in a single sweep from a sensory neurone that responded to noxious stimulation of the skin (N cell⁴). Although there was a slow downward drift in the optical record, the fluorescence increase during the action potential was readily apparent, constituting an effective optical representation of action potentials in this cell. Optical recordings were obtained, as well, in other sensory cells (corresponding to the modalities of touch and pressure) in which the impulses fully invade the soma. The relative intensity change in the experiments on leech neurones was approximately the same as that found with squid axons ($\Delta I/I_r \sim 10^{-3}$),

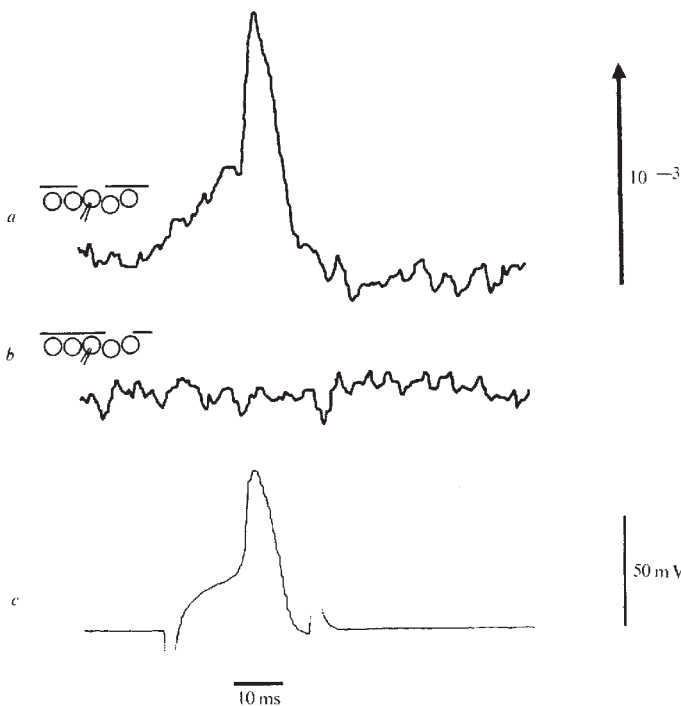


Fig. 2 Measurement of fluorescence intensity (*a*) when the pinhole in the objective image plane was over the image of the stimulated cell, and (*b*) over nearby cells. When the pinhole was moved the equivalent of 95 μm on the ganglion, no intensity change was measured, indicating that the light from the stimulated cell was not significantly spread in the objective image plane. The measurement recorded in trace *b* was made before the measurement recorded in trace *a*. Eight sweeps were averaged in each. The response time constant of the light measuring system was 1.6 ms. Nociceptive cell, 50 μm in diameter. Temperature, 21°C.

suggesting that the merocyanine dye might also be useful as a probe of potential in excitable cells from other species.

Two control experiments ruled out possible artefacts. In the first, the incident illumination was restricted to wavelengths longer than 640 nm, which are not absorbed by the dye. As expected, no intensity change could be detected with this red incident light, while light of the appropriate wavelength (540 nm) resulted in an obvious signal. This demonstrated that the observed effect was a fluorescence change and not, for example, a light scattering phenomenon¹. This result also eliminated the possibility of an artefact resulting from electrical coupling between the systems measuring light intensity and potential. It was also important to show that the light from adjacent cells was satisfactorily separated in the objective image plane, that is, that light reaching the photodetector derived mainly from the stimulated cell, rather than its neighbours in the ganglion. In the experiment shown in Fig. 2, fluorescence was measured (*a*) when the pinhole in the objective image plane was over the image of the stimulated cell, and (*b*) when the pinhole was moved the equivalent of 95 μm in the object plane. There was an optical signal only when the pinhole was positioned over the active cell, indicating that the signal from an individual cell was localised at the cell's image, and was not detectable in adjacent regions of the image plane.

The experiments illustrated in Figs 1 and 2 demonstrate the feasibility of optical monitoring of neuronal activity in one cell with one photodetector. We expect that it will be possible to expand the system to employ an array of detectors so that many neurones can be studied simultaneously. The ability to record activity in many cells at once would allow the construction of a detailed map of the functional connections within a ganglion, and, with additional experiments, should enhance our understanding of the neuronal basis of behaviour.

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Reconstruction of Images from Transforms by an Optical Method

THE holographic reconstruction of the amplitude and phase maps for a bovine liver catalase crystal, by using computer analysis of the image and diffraction pattern obtained in an electron microscope, was reported recently¹. This method used a random number generator to generate an arbitrary phase distribution, and hence to produce from the electron transform a 'first fit' image. This was then filtered by convolution with the image obtained in the electron microscope. We have been attempting to reconstruct images from transforms by an analogue method, using a Rank Precision Industries Image Analyser 3000 to process the images rather than a digital computer. The method which we have defined uses a random spatial frequency generator to produce a 'first fit' image which can be further improved by filtering in reciprocal space. As only the original transform has been used in reconstruction of the image, a task in optics that hitherto has been considered impossible seems to have been achieved (compare Gerchberg¹, where the digital procedure used for reconstruction from the transform, although it used both a transform and an image, worked even when a 'blank' image was supplied).

The use of spatial filtering techniques in optical diffractometry is well known, and is frequently used to improve the signal to noise ratio of micrographs that contain periodic structures. When spatially filtering, the amplitudes of the various components of the Fourier transform of the specimens are modified so that upon recombination, periodic structures in the image are enhanced. If the amplitude filter (spatial filter) placed in the transform plane of the diffractometer is an inverse of the transform of the specimen being examined, then an image will be reconstructed from a 'white' spectrum of frequencies if the phase distribution can be specified.

The phase of the wavefront reaching the transform plane is dependent upon both the physical dimensions of the diffractometer used and the distribution of intensities in the specimen being examined. An attempt was made to synthesise an approximated wavefront at the transform plane and to spatially filter it to reconstruct an image of the

specimen from which the spatial filter had been obtained (in the same diffractometer).

Assuming that the specimen being examined is optically flat (precautions of this nature are usually taken³), then to a first approximation the phase distribution in the transform plane should be a function only of the diffractometer, for a specimen which is small in comparison with the camera length. If the specimen being examined in the diffractometer is a random frequency generator, then a phase distribution of the desired type could be produced.

The construction of a suitable random frequency generator presents a number of problems, and in practice an optical spectrophotometer cell containing a colloidal dispersion was used. Experiments were carried out using this random frequency generator in the specimen plane of the diffractometer to illuminate spatial filters in the transform plane: the light passing through the spatial filter was recombined and examined for periodic structure and it was found that an approximation to the images from which the spatial filters had been obtained appeared (Figs 1 and 2).

These 'first fit' images that were reconstructed from the spatial filters were then positioned in the specimen plane of the diffractometer and spatially filtered using the same filter from which each image was reconstructed. This spatial filtering improves the image quality and reduces the effect of any faults in the random frequency generator. The use

of spatial filtering to improve image quality could in principle be used numerous times. In practice, however, it was found that the necessary processing began to degrade the image after one cycle of the filtering process and no better quality was obtained.

Figure 1*a* shows the original specimen and Fig. 1*b* its optical transform. The halation from the d.c. spot was reduced photographically before the transform was used in conjunction with the random frequency generator to reconstruct the image. Fig. 2*a* shows the reconstructed image and Fig. 2*b* is its transform.

Experiments were carried out to prove that the reconstruction was not caused by a holographic effect³. This was achieved by blocking out the undiffracted light (which could act as a reference beam) at the transform plane of the diffractometer before it reached the spatial filter being used for reconstruction. Figure 3*a* shows the image produced by this method. Examination of its transform indicates that high order information is not reconstructed as effectively as with the d.c. spot present. The image produced by this method is, however, clearly recognisable, and it can be improved considerably by further spatial filtering; Figs 4*a* and 4*b* show poor quality image improved by this method.

Further work is being carried out to improve the quality of the random frequency generator and the techniques of repeatedly spatially filtering the reconstructed image.

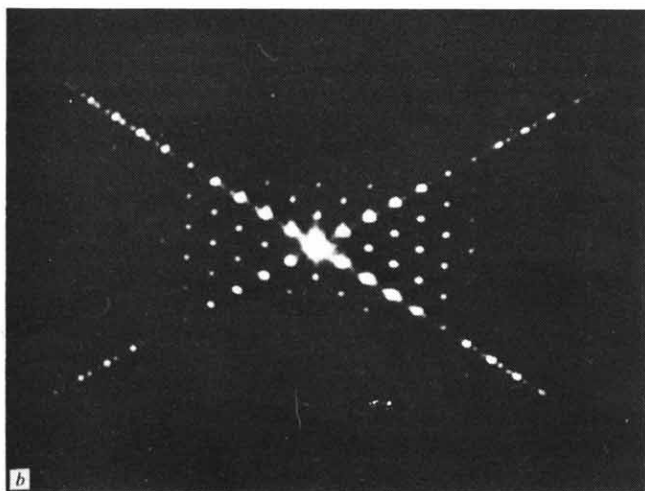
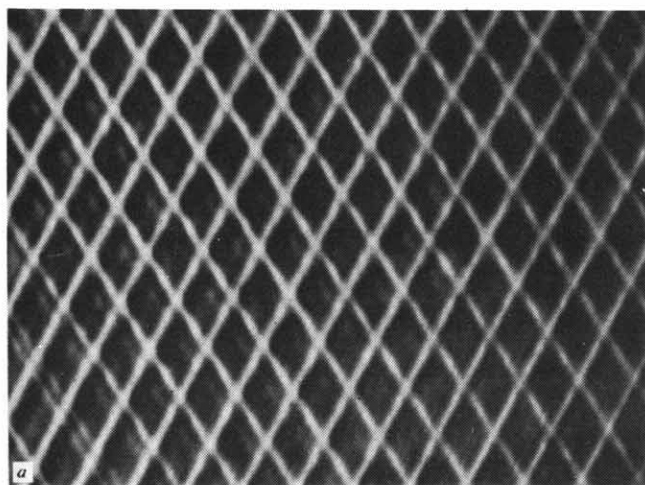


Fig. 1 *a*, The original specimen used in reconstruction experiments, photographed after spatial filtering through the complement of its optical transform. This image is, therefore, the image which would be obtained if a perfect reconstruction, limited only by the quality of the spatial filter, were to be achieved. *b*, Optical transform of the original specimen.

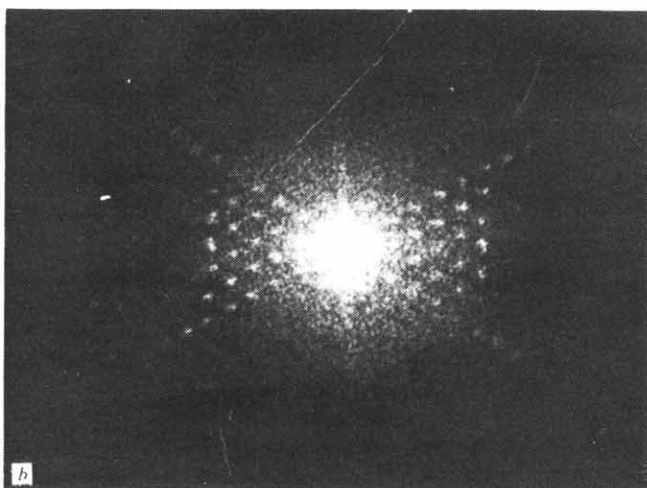
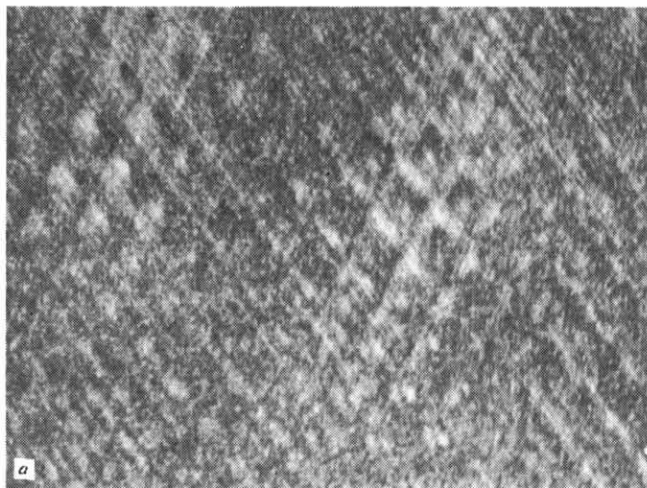


Fig. 2 *a*, A 'first-fit' reconstructed image produced by the procedure defined in the text. *b*, Optical transform of the 'first-fit' reconstructed image. Comparison with Fig. 1*b* indicates a very close relationship, with the strengthening of the d.c. spot and low frequencies indicating a degree of introduced disorder.

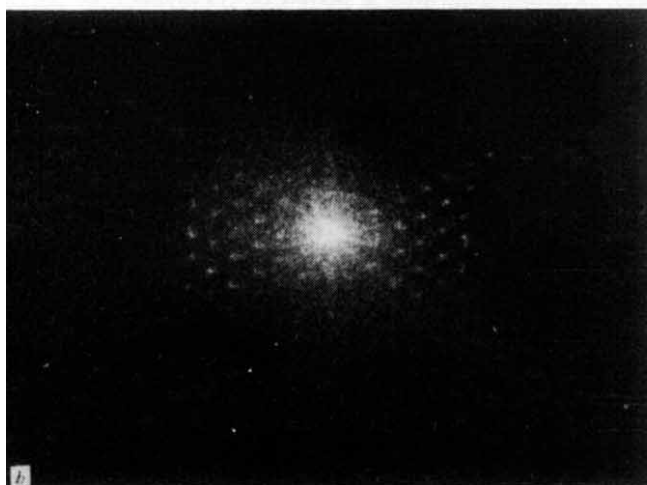
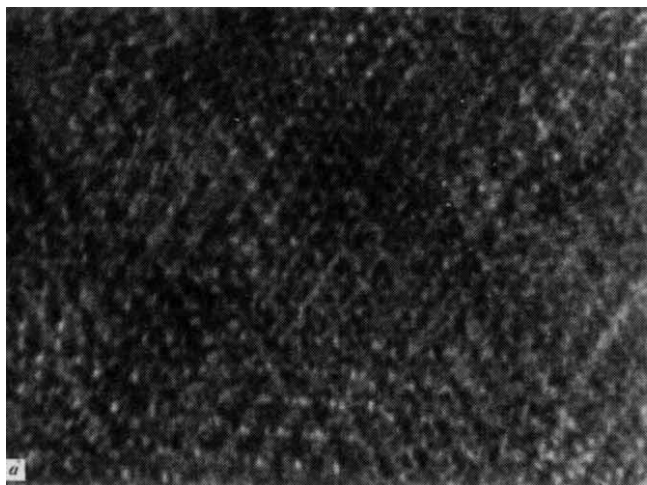


Fig. 3 *a*, A 'first-fit' reconstructed image produced with a stop in the spatial filter plane to eliminate completely the undiffracted beam. *b*, Optical transform of this image. Although the spots are weakened, as compared to Fig. 2*b*, no significant features have been lost.

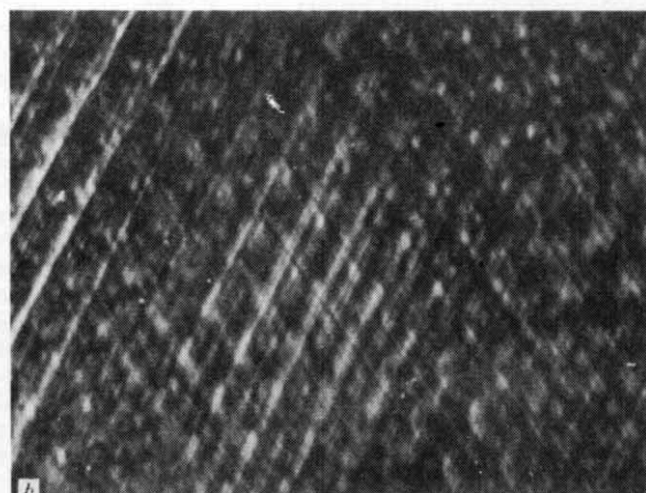
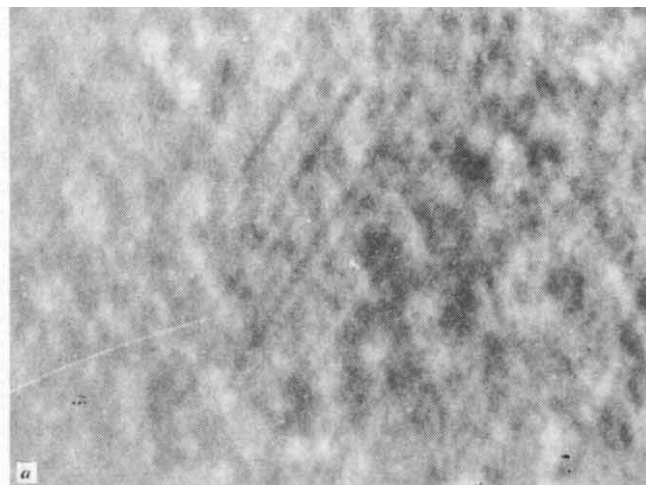


Fig. 4 *a*, A 'first-fit' reconstructed image of rather poor quality, probably due to granularity (visible in the image) in the random frequency generator. *b*, A 'second-fit' reconstructed image, produced by placing the 'first-fit' image in the specimen plane of the diffractometer, with the complementary filter still in position.

Attempts are being made to apply the method to the reconstruction of images from electron and X-ray diffraction patterns.

The ability to reconstruct images from a spatial filter and a random image indicates that extreme care must be taken when using spatial filtering methods to improve the quality of very noisy images, or an artefactual structure defined by the spatial filter may be generated in the reconstructed image, no matter whether it was present or not in the original specimen. Clearly, a 'noisy' electron micrograph can act as a random frequency generator, and the use of a spatial filter which is at all an accurate inverse of a suspected structure is to be avoided. Other hazards of spatial filtering have recently been reviewed⁴. The explanation suggested above for the appearance of reconstructed images is a tentative one, and requires further detailed study to prove or disprove it. The basic phenomenon is, however, readily demonstrated.

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Endocrine Activity retained in Diapause Insect Larvae

DIAPAUSE is a state of arrested development which synchronises insect activity cycles with seasons when food resources are available. Facultative diapause occurs in response to environmental cues such as photoperiod and temperature which influence the endocrine system before the unfavourable conditions arrive^{1,2}. We have now obtained evidence that the currently accepted theory of humoral regulation of larval diapause should be revised. Past research on the pupal diapause of Saturniid moths led to the widely accepted conclusion that larval and pupal diapause result from a failure of the neurosecretory cells of the brain or the corpora cardiaca to release the brain (prothoracotropic) hormone which is required to stimulate the prothoracic glands to secrete the moult-

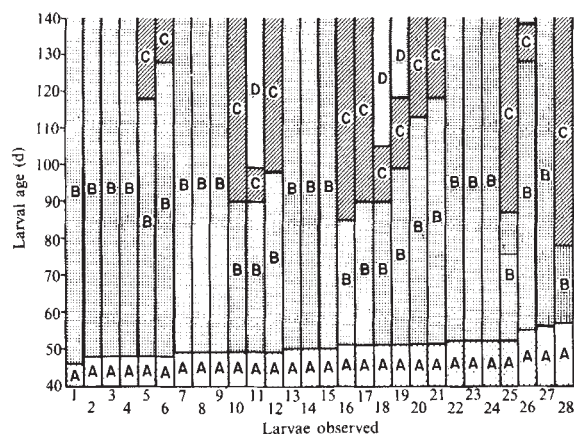


Fig. 1 Bar chart illustrating the occurrence of ecdyses during diapause of twenty-eight mature larvae of *Diatraea grandiosella* held at 23° C; 12 h light, 12 h dark cycle. A, Mature spotted sixth stage larvae; B, immaculate larva; C, immaculate larva which has undergone one ecdysis; D, immaculate larva which has undergone two ecdyses. Under these conditions larvae begin to pupate around 140 d.

ing hormone, ecdysone³⁻⁵. The arrest of growth and moulting and the initiation of diapause have therefore been attributed to the absence of this brain hormone from the haemolymph⁶. Available evidence linking larval diapause with an inactive endocrine system has, however, remained inconclusive⁷⁻¹⁰. Our data indicate that, on the contrary, mature diapause larvae of the southwestern corn borer, *Diatraea grandiosella* Dyar, must retain a functional endocrine system since they undergo stationary ecdyses during diapause. The ability of diapause larvae to undergo stationary ecdyses remains consistent with the concept of diapause as a state of arrested development because the stationary ecdyses do not affect the developmental stage¹¹. (Lüscher used the term stationary ecdysis to describe the ecdysis of the pseudergate larvae of the termite, *Kaloterms flavicollis*, when no progressive development occurs.) We now have evidence to implicate juvenile hormone (JH) in the regulation of this larval diapause.

A Missouri population of *D. grandiosella* which under natural conditions enters diapause in September and pupates in late spring was used for this study. The borers were reared on an artificial diet at 23° C under a 12 h light, 12 h dark photoperiod¹². Under these conditions they passed through six larval stages, reached their mature larval stage around 40 d, and entered diapause. The larvae were then individually transferred to cotton-plugged glass vials containing moist paper strips and maintained under the same regime. Diapause development occurred at a low rate. Pupation began around 140 d, reached 50% around 185 d, and was completed in 210 d. Laboratory-reared and field-collected diapause larvae respond similarly¹³.

A series of randomly-selected larvae was individually observed daily from 40 to 140 d to determine the extent of their moulting activity (Fig. 1). The results show that over a 10 d period between 45 and 55 d each mature larva underwent a stationary ecdysis from a spotted to an immaculate form. This transformation identifies the onset of diapause since the immaculate mature larva which lacks cuticular pigmentation is this borer's distinct diapause form¹⁴. The data also show that after reaching the immaculate stage 50% of the larvae underwent one further stationary ecdysis and 14% two further stationary ecdyses. This phenomenon has been observed in at least 1,500 laboratory-reared and field-collected diapause larvae over the past 2 yr. Each ecdysis which occurred during diapause was typically larval-larval and all larvae retained their immaculate form. Head capsules had a mean width of 2.71 ± 0.05 and 2.56 ± 0.05 mm ($n=10$) before

and after the first immaculate-immaculate ecdysis, respectively. These data yield a ratio of 0.95, which shows that a stationary larval ecdysis had occurred.

According to current thinking, diapause insects are not able to ecdyse. Since our results show that diapause larvae do retain their capacity to undergo stationary ecdyses, even though no growth and differentiation occur, it is important to confirm that the species exhibits a true larval diapause. All the ecological and physiological characteristics of a facultative mature larval diapause are present in diapause larvae of *D. grandiosella*¹⁵. Southwestern corn borers only enter diapause during the mature larval (prepupal) stage, remain inactive, and do not feed. Diapause is induced by simultaneous exposure to low temperatures (<26° C) and short day lengths during the immature larval stages. Although a period of chilling is not required before morphogenesis can be resumed, exposure to warm temperature (>23° C) and long day length accelerates diapause termination¹⁸. Dormancy is therefore genetically programmed and does not occur in direct response to adverse environmental conditions and can only be terminated after a period of reactivation^{1,2}. These traits indicate that this arrested development is a diapause.

The borer has also been shown to exhibit typical physiological adaptations for diapause. In common with other diapause insects, diapause southwestern corn borers have a low respiratory and metabolic rate¹⁶. The oxygen consumption of diapause and mature non-diapause larvae (six replicates of seven larvae each) was measured at 23° C. Newly diapaused larvae were found to have an oxygen consumption of 0.85 ± 0.04 which fell to 0.45 ± 0.01 in mid-diapause, compared with 2.32 ± 0.11 μ l per h/per mg dry weight in non-diapause larvae. The respiratory rate of mid-diapause larvae is therefore about 20% of that of non-diapause larvae¹⁷. Partial dehydration and increased cold hardiness, characteristic of larval diapause^{18,19}, are also found in the southwestern corn borer¹⁸. Ten days after the spotted-immaculate ecdysis, diapause larvae have a mean water content of only 65% (ref. 14).

Metabolic preparations for diapause, which have already been well documented in other species¹⁶, also occur in the southwestern corn borer. Immature larvae have been found to prepare for diapause by accumulating massive triglyceride reserves in their fat bodies; larvae which have just entered diapause in fact contain about three times as much fat body lipid reserves as non-diapause mature larvae^{20,21}. Metabolic preparation for diapause can also be detected in the gonads; spermatogenesis has been found to occur at a much lower rate in pre-diapause immature larvae than in non-diapause ones²². This evidence therefore proves that *D. grandiosella* exhibits a genetically-programmed larval diapause.

Our findings indicate that the humoral regulatory mechanism of diapause southwestern corn borers differs from the one widely accepted as operating in larval diapause. Histological experiments have shown that larvae of *D. grandiosella* contain an endocrine system composed of median and lateral neurosecretory cells, two corpus cardiacum-allatum complexes, and prothoracic glands comparable with those of other lepidopterous larvae. Since such a high percentage of diapause larvae undergo stationary ecdyses, they must retain an active endocrine system and a high titre of JH because larval-larval ecdyses could not occur without its presence. We have obtained specific information about the humoral relationships of diapause southwestern corn borers. Although detailed results are presented elsewhere²³, the main findings can be summarised as follows: (1) Injecting early and mid-diapause larvae with 20-hydroxyecdysone resulted only in stationary larval ecdyses, showing that a lack of ecdysone does not cause diapause. (2) Neck ligations performed on larvae which had just entered diapause resulted in a premature termination of diapause and pupation of the thoraco-abdominal section, showing that a cephalic factor is necessary for the maintenance of diapause. (3) Injecting 20-

hydroxyecdysone into the thoraco-abdominal section of previously-ligated diapause larvae resulted in a premature termination of diapause and pupation, suggesting that ecdysone only terminates diapause when the cephalic factor is absent. (4) Topical treatment with a JH mimic caused non-diapause mature larvae to become immaculate and enter diapause, suggesting that the JH titre regulates diapause induction. (5) The periodical topical application of a JH mimic to diapause larvae prolonged diapause and increased the number of stationary larval ecdyses, suggesting that the JH titre regulates the maintenance of diapause. We conclude that JH which is produced by the corpora allata in the head is the principal regulatory agent for this larval diapause. Although other investigators have proposed a role for JH in the regulation of larval diapause²⁴, this study has provided the first direct evidence to support its involvement.

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Dislocations in Microtubular Bundles within Spermatozoa of the Coccid Insect *Neosteingelia texana* and Evidence for Slip

THERE is considerable evidence that movement of cilia and flagella depends on slip or sliding between tubules¹⁻³. The evidence, however, is confined largely to cilia and flagella with the common arrangement of a central pair of microtubules surrounded by nine double tubules, the '9+2' arrangement. Coccid insects have filamentous spermatozoa with motility much like flagella and yet they have an arrangement of microtubules quite different from the 9+2 type⁴⁻⁸.

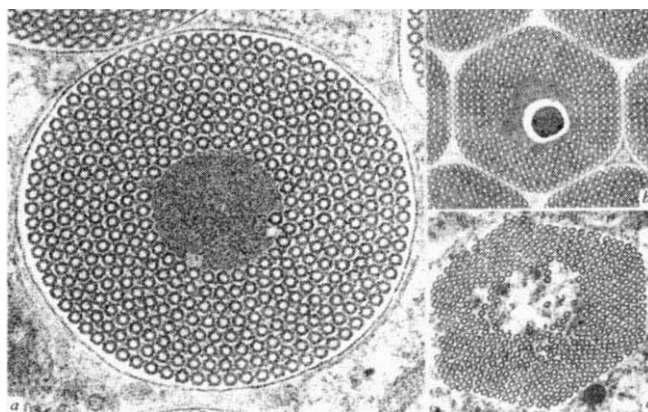


Fig. 1 Cross sections through sperm of *Neosteingelia texana*. *a*, Microtubules arranged in rings in an unbundled sperm $\times 78,200$. *b*, Hexagonal microtubular pattern in a sperm tightly packed in a bundle $\times 35,000$. *c*, Hexagonally arranged microtubules in a disintegrating sperm from a dead insect $\times 35,000$.

Although there is reason to believe that slip between microtubules may be involved in morphogenesis of the corkscrew-shaped region of sperm bundles of the mealybug *Pseudococcus obscurus*⁹, apparently no evidence of slip in movement of coccid sperm has been reported.

The type of slip described in cilia and flagella of the 9+2 type is localised or caterpillar-like¹⁻³. This may be of the same kind as occurs between layers of atoms in a deforming crystal¹⁰. The localised regions at which slip occurs in crystals are known as dislocations. Gliding dislocations, at which slip is occurring locally, have been suggested as an important common denominator of many types of movement at the cellular level¹⁰⁻¹².

Analysis of cross sections of sperm from *Neosteingelia texana* shows that dislocations can be identified in the array of microtubules that make up the bulk of the cell. The pattern of dislocations is not static but depends on the state of the sperm. This observation provides what appears to be the first clear evidence of movement of dislocations at the subcellular level. The movement of the dislocations implies localised slip between microtubules. The type of slip, however, differs from that described in the common form of cilia and flagella: the microtubules slide across one another rather than along one another. This is not to say that there can be no longitudinal slip. It is quite possible that movement involves both kinds.

Fig. 1 shows cross sections of microtubular bundles in three states. Experimental methods and materials are described elsewhere⁸. Developing sperm group into bundles which become motile. While still in the free state a sperm appears round in cross section as shown in Fig. 1*a*. When the sperm becomes tightly packed into a bundle its outline is roughly hexagonal as in Fig. 1*b*. Six radial lines at the corners divide this microtubular array into six symmetrically equivalent regions. The microtubules lie on concentric hexagons with rounded corners. A third state occurs when sperm development is interrupted by premature insect death from natural causes. Sperm which normally would be round become hexagonal during their disintegration as shown in Fig. 1*c*.

Except near the six boundaries in Fig. 1*b* the microtubules are arranged on a lattice that is rectangular and nearly square. As one approaches a boundary the packing becomes irregular. Crossing the boundary the array becomes rectangular again but with a different orientation. The disintegrating sperm of Fig. 1*c* shows a similar, if less ordered, arrangement. Boundaries separating lattices of different orientation are known in the literature of crystal physics as grain boundaries. The simplest interpretation of the boundaries in Fig. 1*b* and *c* is that they are radial planes

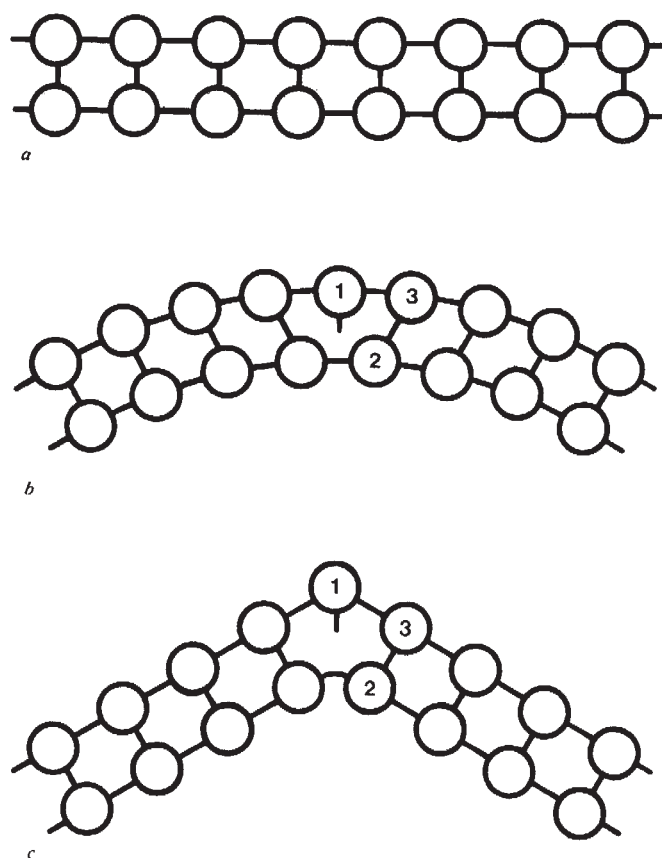


Fig. 2 A perfect and strain-free array of microtubules is shown in (a). Strain in an array of microtubules lying on two concentric circles with a dislocation situated at microtubule 1 is shown in (b). c, Polygonised array with strains each side of the dislocation relaxed. The dislocations in (b) and (c) move as follows: the link from 2 to 3 breaks and forms with 1. The dislocation is then situated at 3, having moved one space to the right.

that separate lattices differing in orientation by a rotation θ about a longitudinal axis. Such boundaries are termed simple tilt boundaries¹³. For perfect hexagons θ would be $\pi/3$ rad. Similar tilt boundaries are found in the arrays of microtubules in heliozoan axonemes¹⁴.

Along the axis of each sperm in Fig. 1 lies a wedge disclination of rotation 2π rad^{10,15}.

A superficial examination of the arrangement of microtubules in Fig. 1a reveals no boundaries. A careful analysis of the pattern of dislocations, however, shows that the boundaries are indeed there and that the pattern of Fig. 1a is simply related to those of Fig. 1b and c.

The neighbouring rings of microtubules in these sperm usually exhibit a difference of six microtubules (Fig. 1a, Table 1). Microtubules arranged at regular intervals in two rows can be matched in pairs if the rows are straight (Fig. 2a) but not if they are curved (Fig. 2b). In the first case the rows may be said to match perfectly all along their length but in the second case the rows match perfectly at the ends only. As one follows the rows away from the ends in Fig. 2b, one finds increasing mismatch or strain until in the middle one finds a region of maximum mismatch. The region of maximum mismatch represents an edge dislocation. The top row has one more microtubule than the other and the extra microtubule may be viewed as being located at the dislocation. The dislocation is a line of maximum mismatch running along the bundle and seen in cross section as a point of maximum mismatch.

Extending the two rows in Fig. 2b repeats the pattern and finally results in a pair of concentric rings. Regions

Table 1 Number of Microtubules in Consecutive Rings from the Most Peripheral (No. 1) to the Most Central Complete Ring in Cross Sections from the Posterior Regions of Forty Sperm

No. of sperm	Microtubules per ring							
	1	2	3	4	5	6	7	8
1	47	41	36	30				
1	47	41	35	30	23			
2	48	42	36	30	24			
1	54	48	42	36	30			
1	54	48	42	36	29			
1	55	49	43	37				
1	57	51	46	39	33			
1	60	52	47	42	36			
1	66	60	54	48	42	36	30	
3	71	65	59	53	47	41	35	
1	72	66	60	55	49	42	37	31
4	72	66	60	54	48	42	36	
5*	73	67	61	55	49	43	37	31
6	73	67	61	55	49	43	37	
1	73	67	61	55	49			
4	74	68	62	56	50	44	38	
3	75	69	63	57	51	45	39	
1	75	69	63	57	51	45		
1	78	72	66	60	54	48	42	
1	79	72	66	60	54	48	42	35

* The sperm cross section of Fig. 1a fits in this group.

of perfect match alternate with regions of maximum mismatch. The number of regions of maximum mismatch and of perfect match is the difference in number of microtubules between the two rings. One therefore expects to find six dislocations equally spaced at angular intervals of $\pi/3$ rad, corresponding to the observed difference of six microtubules.

Freed of all externally applied stresses the two adjacent rows of Fig. 2b are expected to relax into the kinked arrangement of Fig. 2c. The rows now match perfectly everywhere except at the dislocation. Complete rings will become polygons with dislocations located at the corners. In particular if there are six dislocations the polygons will be hexagons. Such a kinked arrangement represents the simplest form of what in crystals is termed polygonisation, in which arrays of dislocations are found at the kinks (ref. 15, page 40).

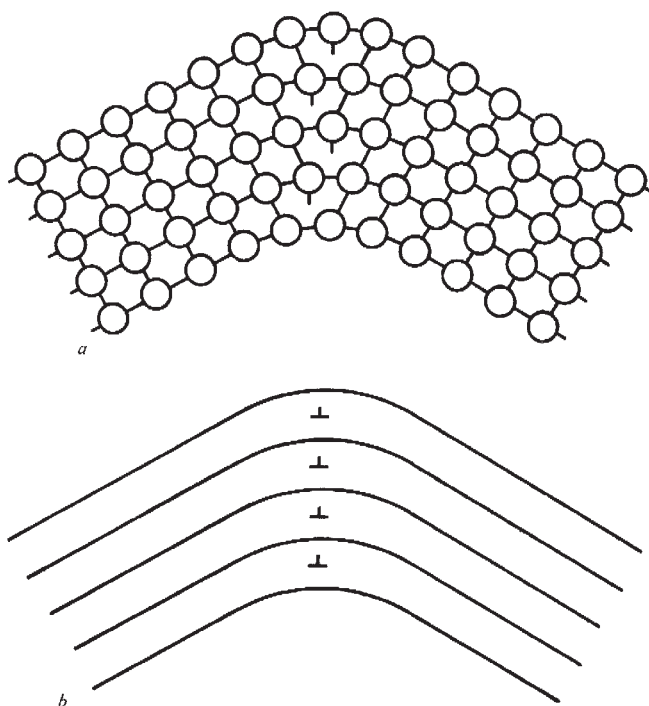


Fig. 3 An idealised view of the arrangement of microtubules (a) and dislocations (b) at corners in Fig. 1b and c. The dislocations lie on radial boundaries.

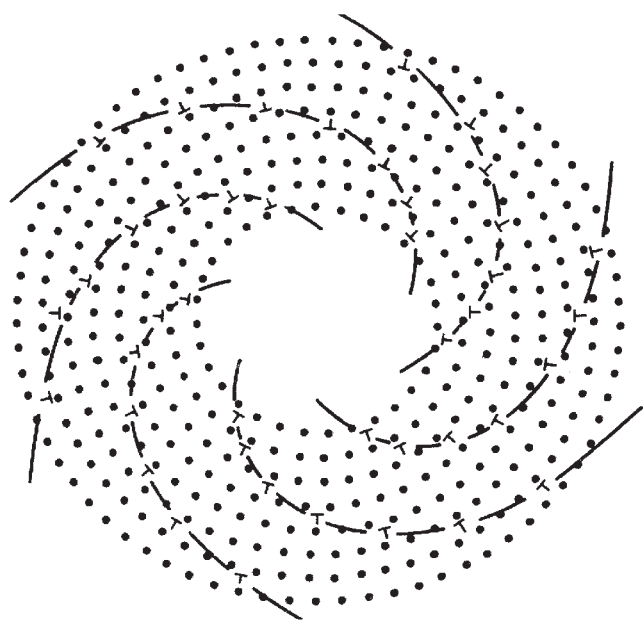


Fig. 4 A tracing of the arrangement of dislocations in the sperm of Fig. 1a. The radial boundaries of Figs 1b and 3 are distorted into six spiral boundaries at angular intervals of approximately $\pi/3$ rad.

The edge dislocations of Fig. 2b and c may glide along the rows by breaking and making inter-row links in a cyclic fashion. For example, if the link from microtubule 2 to microtubule 3 is broken at 3 and joined to microtubule 1, then the dislocation will have glided one space to the right and be situated at microtubule 3. Repeating the process moves the dislocation further to the right and results in local, caterpillar-like slip of the top row one space to the right relative to the other row. The amount of slip occurring at a moving dislocation is given by the Burgers vector of the dislocation. Presumably such a process of making and breaking links would require the consumption of ATP or some related compound.

Dislocations arranged as in Fig. 3 form a simple tilt boundary. This is an idealised picture of the radial boundaries in Fig. 1b and c. Careful examination of the matching between rings in Fig. 1a reveals dislocations lying on six spirals (Fig. 4). The radial boundaries of Figs 1b and 3 have become the curved boundaries of Figs 1a and 4. The rectangular lattice between boundaries has become strained as in Fig. 2b. As sperm disintegrate in dead insects the strain is lost, the curved boundaries straighten and the cross section regains its hexagonal appearance (Fig. 1c).

Change in shape of the boundaries implies movement of individual dislocations making up the boundaries. Slip of microtubules across one another occurs locally at the moving dislocations.

The above observations provide no direct evidence of movement of dislocations and slip occurring between microtubules during movement of an active spermatozoon. They show only that these processes accompany transition of the sperm from a free state to one of tight packing within bundles, and from an active to an inactive, disintegrating form. In general, dislocations distort the shape of rod-like structures that contain them¹⁶. It is possible that the shape changes required for the movement of these coccid sperm are due to moving dislocations in their bundles of microtubules. The dislocations may glide circumferentially in an active sperm and account for its motility. A similar suggestion for the movement of bacterial flagella is made elsewhere¹². Movement of the dislocations could be driven by the local consumption of ATP in making and breaking links

between microtubules in the neighbourhood of the dislocations.

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Assembly Pheromone(s) in the Soft Tick *Argas persicus* (Oken)

PREVIOUSLY no pheromones have been discovered in soft ticks, although a few have been demonstrated in hard ticks¹⁻³ and hundreds in insects⁴. We have now shown that both males and females of the soft tick *Argas persicus* produce material(s) to induce aggregation, which could therefore be considered as assembly pheromone(s) (Fig. 1), acting both within and between the sexes.



Fig. 1 Mass assembly of *Argas persicus* clinging to a paper containing pheromone. Male ticks moved under the paper which had female pheromone to aggregate in this position within an hour. The entire assembly was suspended by forceps for the photograph (about $\times 6$).

This assembly pheromone was demonstrated by the following general procedure (for example, Fig. 2). We placed disks of filter paper for 2 or more days in the bottom of a jar with unmated female ticks. Other disks were treated in the same way with unmated males. Petri dishes (14 cm in diameter) were marked off into eight sectors, each containing a 2 cm disk of filter paper. To assay a test disk, we placed ten blood-fed virgin ticks in the centre of a dish with its eight surrounding disks, treated or untreated according to the experiment. We then transferred the plate to a dark incubator at 30° C and 49% relative humidity to approximate optimum natural conditions. Periodically the position of the ticks was observed through a window covered with red cellophane.

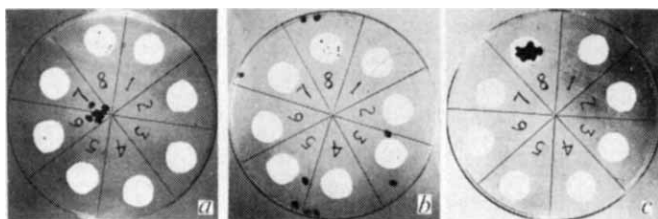


Fig. 2 Assembly activity in ticks which were placed in the centre of the dish and photographed from below after various intervals: (a) 1 min; (b) 3 min; (c) 30 min. Disk 8 had pheromone.

We first examined the response of male ticks. We prepared ten dishes each with eight untreated disks, and ten others containing seven untreated and one treated disk: each dish contained ten ticks. After 1 h in dishes in which all eight papers were untreated, the males were dispersed, singly or in small groups, in a pattern not significantly different from that expected for a binomial distribution ($\chi^2=5.52$, 3 d.f., $P>0.10$). Any tendency for the ticks to form clusters (to reassemble) was not measurable for several hours. χ^2 tests also indicate that the ticks showed no significant preference for any of the sectors ($\chi^2=12.13$, 7 d.f., $P>0.05$).

However, when one sector (No. 8) contained a disk with female pheromone there was a very highly significant change ($\chi^2=84.0$, 3 d.f. with $P<0.001$) in the distribution of the ticks. Within 1 h 82.2% of the ticks were assembled in sector 8, crowded beneath the disk treated with female pheromone.

To test the male tick's response to organic matter of arthropod origin a fruit fly or mosquito was crushed and spread on filter paper. In no case was assembly stimulated.

We further found that males of *A. persicus* respond rapidly to the pheromone(s) of both sexes (Fig. 3). For these experiments plates contained seven untreated disks and one with either male or female pheromone. Within the first hour of the test period an average of 75% of the male ticks were under the paper contacted by male ticks and more than 80% were under disks contacted by females.

Females respond more slowly than males regardless of the sex from which the pheromone is obtained (Fig. 3). Within the first half hour no assembly was manifest: only after 2 h was aggregation indicated. These treated papers to which females were relatively unresponsive were again used with males which showed no appreciable change from their assembly as noted above. Thus, the slower aggregation of females appears characteristic of the sex.

The greater activity of the males and their rapid response to the pheromone(s) agree with the observations on hard ticks. Males actively seek females of the lone star tick *Amblyomma americanum* (Linnaeus)^{1,2,5}, the Gulf Coast tick, *A. maculatum* Koch, and the American dog tick, *Dermacentor variabilis* (Say)².

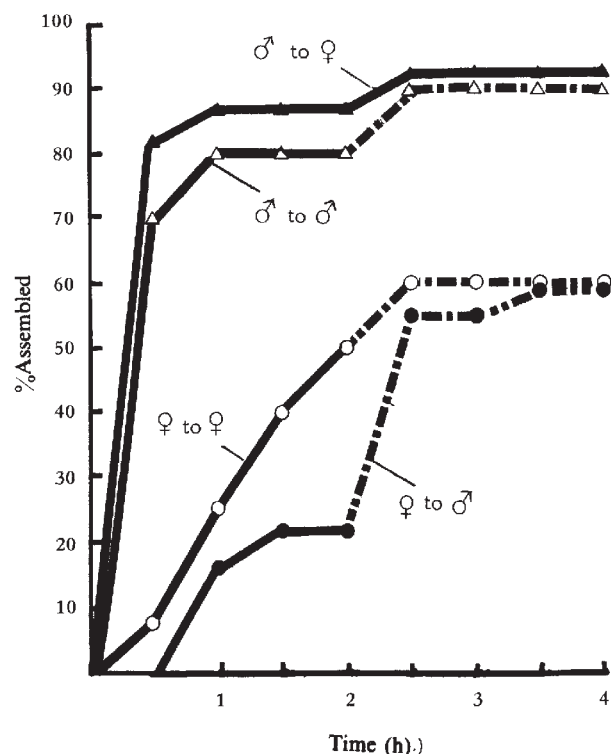


Fig. 3 Rate of assembly of ticks under a previously contacted test disk. Solid line represents results of tests of forty to fifty ticks; dotted line represents results of tests on ten to twenty ticks. $\blacktriangle$, Male ticks assembling under a female-contacted disk; $\triangle$, males assembling under a male-contacted disk; $\circ$, females under a female-contacted disk; $\bullet$, females under a male-contacted disk.

The female pheromone could be extracted in saline but not in organic solvents such as ether, benzene or acetone. Saline washings of paper contacted by females were centrifuged and the supernatant transferred to a clean paper disk which was air-dried. A half-hour bioassay of the freshly impregnated disk clearly indicated activity. When similar disks with acetone extracts showed no activity, the original papers from which the extracts had been prepared were again tested. Normal pheromone activity of these papers appeared unimpaired by the treatment. These results indicate that the active material had remained unchanged in the paper during extraction.

Further tests showed that the female pheromone is relatively thermostable. Freezing an active piece of filter paper for 24 h at -16° C and heating it for 5 min in an oven at 100° C did not destroy activity. This latter property enhances the potential usefulness of the pheromone(s) as a lure for trapping this harmful species.

Studies on the source of the pheromone(s) and on the tick's receptors for its detection are in progress.

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Visual Pigment of the Freshwater Stingray, *Paratrygon motoro*

In general the absorbance spectra of the visual pigments of deep-water and pelagic marine animals are blueshifted compared to those of coastal and estuarine animals, and the pigments of the latter are blueshifted compared with those of freshwater animals. This trend, which correlates with the transmittances of the waters, is best documented for the Osteichthyes (bony fishes)¹. The marine members of this Class, except for some wrasses, have visual pigments based on vitamin A₁ (rhodopsins of $\lambda_{\max}$ 473 to 512 nm). In freshwater Osteichthyes, however, the need for longer-wave sensitivity is met by pigments based on vitamin A₂ (porphyropsins of $\lambda_{\max}$ 523 to 543 nm) or by mixtures of porphyropsin and rhodopsin or, in one case at least (the gwyniad²), by a rhodopsin of high $\lambda_{\max}$ (520 nm).

A similar trend of $\lambda_{\max}$ is apparent in the visual pigments of the Chondrichthyes (cartilaginous fishes). For example, Denton and Shaw³ found that the rhodopsins extracted from three deep-sea sharks ($\lambda_{\max}$ 472 to 484 nm) are in the same range as those of deep-sea Osteichthyes (473 to 490 nm), while the extracted rhodopsins of surface-living sharks and rays have $\lambda_{\max}$ = 497 to 499 nm (ref. 1). Until recently none of the very few cartilaginous fishes that inhabit freshwater has been examined in this respect. Consequently, the report⁴ that the intact retina of the Amazonian stingray, *Paratrygon motoro*, absorbs maximally around 510 nm seems to provide a rare parallel to the visual pigments of the freshwater Osteichthyes, and to raise the question whether its pigment is a porphyropsin or a redshifted rhodopsin.

It should be noted, however, that other bottom-living fishes (Osteichthyes) from the same region were found in that investigation⁴ to have retinal $\lambda_{\max}$ 20 to 30 nm longer than *Paratrygon*. Moreover measurements made on intact retinæ may not be strictly comparable with those made on extracts⁵. Thus one cannot be sure that this apparent difference between the pigments of marine and freshwater rays is real. For this reason we examined a conventional extract.

It was made with three retinæ from two specimens of *Paratrygon motoro* (Mueller and Henle) caught in the Rio Negro near Manaus, over 1,000 miles from the mouth of the Amazon. The retinæ were dissected out, frozen and brought to Sussex for analysis. Facilities for keeping them frozen during transport were not good, and they thawed several times, which probably accounts for the small yield of pigment.

The photoreceptor outer segments were obtained as a minute pellet (by Saito's sugar flotation process⁶) which, after washing with neutral buffer, was extracted overnight with 0.5 ml 3% digitonin solution. The extract was made alkaline with sodium borate and 0.005 M in hydroxylamine. The absorbance spectrum showed only a small amount of pigment, insufficient for homogeneity tests. Consequently it was fully bleached by exposure to white light passed through a Wratten 15 filter. The spectral change in absorbance is shown in Fig. 1. The pigment band agrees with a 499-nm nomogram curve and the product band is characteristic of retinal oxime, confirming that the pigment

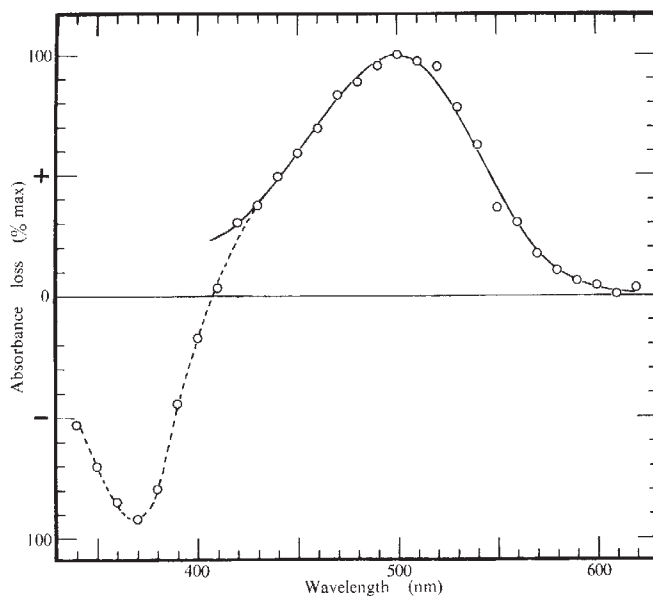


Fig. 1 Normalised difference spectrum (actual $\Delta A_{\max}$ = 0.0115) of the Stingray visual pigment (---) in the presence of 0.005 M hydroxylamine. The product band ($\lambda_{\max}$ ~ 370 nm) is characteristic of the oxime of retinal. —, λ 499-nm 'nomogram' pigment. Optical path length 10 mm; volume of extract 0.55 ml, pH 8.45.

is a rhodopsin. Thus there is no significant difference between the rhodopsins of freshwater and marine rays.

Assuming that the 499-nm rhodopsin is the only pigment of the *Paratrygon* retina, it would seem that this animal is not well adapted to its freshwater environment. Its retina, however, is backed by a gold-coloured tapetum⁴ and this will shift the effective sensitivity to longer wavelengths. Although it is possible, therefore, that the presence of a 'normal' rhodopsin means that neither a porphyropsin nor a redshifted rhodopsin could be evolved in this line, it could also mean that a golden tapetum was a simpler solution to the problem. The colour disappears on addition of glycerol⁴, which suggests that, as in other Chondrichthyes, it is an interference phenomenon⁷. The reflectivity might, therefore, have moved to longer wavelengths, during evolution, by a change in the spacing of the tapetal plates.

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Origin of the European Eel

THE controversy about the identity of the European eel was started by Tucker in 1959¹. Contrary to the theory of Schmidt², which assumed that both European and American eels return for spawning to the Sargasso Sea, Tucker argued that it is unlikely that the eels from the European waters manage to return, and that the eels which spawn in the Sargasso Sea are therefore all American. If this were true, the European eel should originate from the same gene pool as the American eel, and have identical gene frequencies, unless differential mortality changes these frequencies.

Morphological and biological evidence, summarised by Deelder³ and more recently by Sinha and Jones⁴, cannot clarify the genetic identity of the European eel. Differences in the frequencies of electrophoretic variants of proteins between American and European eels have been described⁵⁻⁷. For the discovery of conclusive evidence, the variation detected by electrophoresis should be of genetic origin, and differences between American and European eels should be such that they cannot be accounted for by differential mortality of eels possessing particular genotypes. Koehn⁸ has pointed out that "recent literature suggests that allelic isoenzyme variation is responsive to local environmental conditions" suggesting that 'differential intrageneration survival'¹⁴ should be taken into consideration when differences in gene frequency within the geographic range of a species are evaluated. This could apply particularly to a fish like the eel, which during its long larval life is distributed over a wide geographic area and in its second juvenile stage (as an elver) enters a variety of water systems with different conditions. It therefore remains to be shown whether the differences between American and European eels in the frequency of those characters of which the genetic nature has been established within the possible limits^{5,6} are caused either by genetic isolation of the two groups or by environmental influences.

Table 1 Number of Postulated Transferrin Alleles

	Allele				
	A	B	C	D	2N
Iceland	7	34	24	9	74
Scotland	8	17	49	6	80

$$\chi^2 = 14.73_{d.f. = 3}.$$

The genetic control of the transferrin variants detected by Fine *et al.*⁵ is indicated by their phenotypic distribution⁹. Differences in the phenotypic distribution between American and European eels were found⁵, but differences were also observed between eels from different locations in Southern Europe^{10,11}. To explain the latter finding, Drilhon and Fine¹² suggested that the eels in the Eastern Mediterranean reproduce there and form a separate reproductive group. A gene count analysis of the data of Pantelouris *et al.*¹³ on the transferrins of eels from Scotland and Iceland also shows a significant

difference (Table 1). Phenotypic differences in liver esterases of eel from various northern European locations, observed by the same authors, led them to conclude that regional differences in gene frequency may exist for the European eel and to speculate that these differences "may reflect subdivision of populations of the European eel, but they may also be attributed to differential selection"⁷.

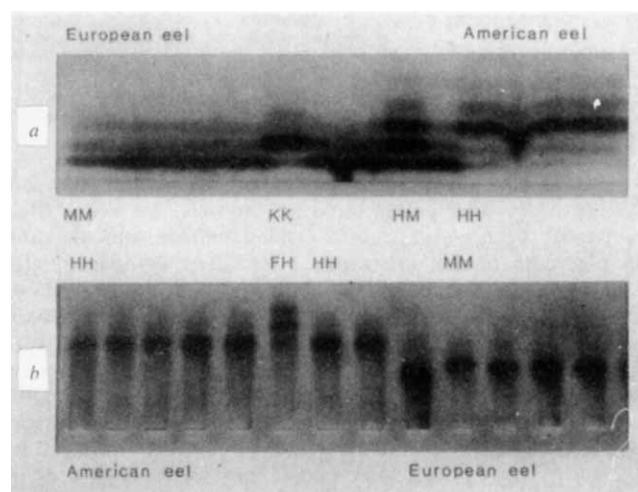


Fig. 1 Livers were homogenised in 1 volume 1% NaCl, buffered at pH 7 and containing 1 mM EDTA, 1 mM MgCl₂ and 1% Triton-X. Homogenates were centrifuged for 15 min at 2,800 r.p.m. and the supernatant was diluted 1:2 or 1:32 for electrophoresis in starch gel or acrylamide respectively. Acrylamide gel slabs were used routinely, but when less well defined patterns were encountered, runs were duplicated in starch gel. *a*, MDH phenotypes in starch gel (11% starch in 0.008 M Tris-citrate, pH 6.7; electrode buffer: 0.223 M Tris-citrate, pH 6.3 (ref. 15); staining fluid: 1,450 mg malic acid, 10 mg NAD, 10 mg 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), 10 mg phenazine methosulphate (PMS) in 100 ml 0.5 M Tris-HCl, pH 8.6 (ref. 16). *b*, MDH phenotypes in acrylamide gel (7% Cyanogum in 0.015 M Tris-citrate, pH 7.0; electrode buffer: 0.15 M Tris-citrate, pH 7.0; staining fluid: 60 mg malic acid, 40 mg NAD, 15 mg MTT, 5 mg PMS in 100 ml 0.05 M Tris-HCl, pH 8.5).

The presence in American eels of a haemoglobin allele, at a frequency of over 3%, that was not found in 748 European eels was considered by Sick⁶ strong evidence against Tucker's one-population concept, unless it is postulated "that in the European part of the population, specimens carrying this allele are exterminated by natural selection".

A recently detected difference in the frequencies of the allozymes of malate dehydrogenase (MDH) between eels from the coast of Maine (USA) and eels from the Azores, Southern Spain, Greece, Holland and Poland now provides conclusive evidence that the eels from the latter five locations are not recruited from the same spawning stock as the eels from Maine. MDH was detected in liver extracts analysed by zone electrophoresis.

Table 2 Observed and Expected Distribution of MDH Phenotypes

Location	FH	HH	HK	HM	HS	KK	KM	KS	MM	MS	MV	N
Poland				3			1		59	7		70
Holland		1		6	2		6		57	3	1	76
Spain			1	8	1	1	3		76	4		94
Greece				4			2	1	53			60
Total (Europe)	1	1	1	21	3	1	12	1	245	14	1	300
		0.6	0.6	24.2	0.8	0.2	12.4	0.4	240.8	16.1	1.1	
Azores				2					22	1		25
Maine, USA	1	63		6								70

Table 3 Frequencies of Postulated MDH Alleles

Location	MDH ^F	MDH ^H	MDH ^K	MDH ^M	MDH ^S	MDH ^V	N
Poland		0.021	0.007	0.921	0.050		70
Holland		0.066	0.040	0.855	0.033	0.006	76
Spain		0.053	0.032	0.888	0.027		94
Greece		0.033	0.025	0.933	0.008		60
Total (Europe)		0.045	0.023	0.900	0.030	0.002	300
Azores		0.040		0.950	0.010		25
Maine, USA	0.007	0.950		0.043			70

Details of the methods and the major phenotypes observed are given in Fig. 1. The patterns consisted of one or three bands, and agreed with those expected for dimeric molecules of which the peptides are controlled by allelic genes at one polymorphic locus. The patterns were labelled according to the relative mobility of the bands, and genetic control by six alleles (MDH^F to MDH^V) was assumed.

The distribution of the phenotypes observed in the various samples is given in Table 2, together with the expected Hardy-Weinberg distribution for the total of the European samples. In Table 3 the frequencies of the postulated alleles are given. It may be seen from this table that MDH^H has a frequency of 0.950 in the sample of American eel, but ranges in the European samples from 0.021 to 0.066. The sample from the Azores, with a frequency of 0.040, fits well with the European samples. No significant differences were observed between the European samples. The data presented show no differential mortality with regard to the MDH types within the European area, ranging from Greece to Poland. The similarity of the MDH frequencies in the eels from the Azores and from Europe shows that no differentiation of the MDH types takes place during nearly half of the total oceanic migration route of the eel larvae either. Therefore it seems highly unlikely that the difference in MDH frequencies between American and European eels could be due to differential mortality caused by environmental factors. Additional studies of a number of enzyme systems, and studies of esterases and serum proteins, to be published elsewhere, reinforce the conclusion that American and European eels are of different parental origins.

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PCB Residues in Plankton from the Gulf of St Lawrence

ORGANOCHLORINE residues in coastal and oceanic plankton have usually been measured using samples taken at irregular intervals and undifferentiated with respect to particle size^{1,2}, so it has not been possible to relate the variance between samples to temporal differences in supply (or uptake) of contaminants or to differences in plankton composition. Here we describe the variation in polychlorinated biphenyl (PCB) levels with particle size and time in Gulf of St Lawrence plankton.

Nine samples were taken at approximately 10 d intervals from June to August 1972 from a station about 16 km offshore from the northern coast of Prince Edward Island. For each collection a standard No. 20 net (0.5 m diameter, 73 μ m mesh width) was rinsed in isopropyl alcohol and then towed for approximately 20 min through the top metre of the water column near the wake of the boat. Samples were washed with seawater through a stack of Nitex^R screens and split into ten size fractions, with the exception of the collection on June 3 which was split into five parts because it contained few small copepods. The particles retained by each screen were stored in glass vials at -20° C for about 12 weeks before analysis. In addition to these samples, the standing crop of the various size fractions of plankton, including any attendant suspended particulates, on each sampling date, except August 27, was estimated from vertical net tows in the study area.

Organochlorine residues extracted with the acetonitrile based system of Onley³ were cleaned and analysed as described previously⁴. Routine GLC analyses were carried out on a glass column (6 inch $\times$ $\frac{1}{8}$ inch internal diameter) packed with 3% Dexsil 300 (Analabs, Inc.) on 100 to 120 mesh Gas-Chrom Q, operated at 240° C. Comparisons were made with standards of the DDT group and PCBs (as Aroclor 1254^R, Monsanto Ltd). The presence of PCBs was confirmed in selected samples (the August 10 series) by behaviour similar to standards in GLC on a glass column (4 inch $\times$ $\frac{1}{8}$ inch) packed with 10% EGSS-X on 100 to 120 mesh Gas-Chrom Q (preconditioned, Applied Science Laboratories) operated at 195° C and by constancy of peak height ratios and retention times following treatment with ethanolic KOH (ref. 5) or CrO_3 (ref. 6). Although peaks were stable to the latter two treatments, there was some overall loss of recovery attributable to holdup in the interface during extraction. Similar behaviour was observed in PCB-free plankton (August 10, large size fraction) spiked with Aroclor 1254.

PCB concentrations were calculated from average height ratios of several peaks in samples relative to those in standards. Peaks were chosen not to overlap with those of the DDT group. Chromatograms of extracts from the smallest size fractions were remarkably similar to the standards both in retention time and in relative peak height; in larger size fractions, the retention times coincided with standard peaks but the relative peak heights were somewhat different. Treatment with ethanolic KOH or CrO_3 failed

to produce significant changes in the PCB peaks overlapping *p,p'*-DDE or *p,p'*-dichlorobenzophenone, indicating that the DDT group residues were too low to be reliably estimated. Minimum detectable levels of *p,p'*-DDE, *p,p'*-DDT and PCB using these methods were approximately 0.01 to 0.1, 0.02 to 0.2, and 0.2 to 2 p.p.m. wet weight, respectively, depending on sample size.

The possibility that the samples became contaminated during collection can be discounted for two reasons. First, hexane extracts of net material, sampling gear, boat paint (deck and anti-fouling paint), bilge water, and seawater from the wake were free of PCBs. (The anti-fouling paint was particularly suspect¹, but our analyses were confirmed by the manufacturer who assured us that PCBs were not used during its preparation.) Second, residue levels were statistically independent of the standing crop of plankton in the water column and the amount of material analysed ($P > 0.05$).

and previous estimates can be attributed to differences in mesh size of the collecting net. For example, if we assume that the widely used No. 6 plankton net will fail to catch particles less than the nominal mesh size, 239 μm , but will retain larger particles in the same proportion as a No. 20 net, our results suggest that No. 20 net samples (Table 1, A) will yield, on average, about twice the residues calculated to be present in an equivalent mass of No. 6 net plankton (Table 1, B). Of course these figures are only approximate and will vary according to the composition of the plankton. Nonetheless, our estimates of the probable PCB content of No. 6 net samples are comparable with those found by Risebrough *et al.*².

If the body size/concentration relation found here is applicable to particles smaller than 73 μm , then it seems that even calculations based on No. 20 net samples still underestimate the actual concentration of organochlorines in marine plankton. Cox⁷ seems to have reached a similar

Table 1 PCB Concentrations (p.p.m. Wet Weight) in Gulf of St Lawrence Plankton

Size (μm)	June 3	June 29	July 9	July 17	July 24	August 1	August 10	August 19	August 27
73		7.63	17.06	2.28	7.08	0.68	20.8	3.47	0.41
102	31	6.53	22.12	0.72	1.30	0.37	3.18	1.53	0.15
153		14.43	8.26	0.63	1.01	0.34	8.28	1.95	0.39
202		8.50	7.90	1.45	0.71	ND	0.90	8.55	0.89
253	1.7	1.98	1.95	1.34	0.69	ND	0.21	1.74	ND
308		5.53	3.33	0.67	3.53	ND	0.23	4.73	ND
351		0.29	ND	0.70	1.32	ND	0.27	0.62	ND
505	5.5	0.15	ND	ND	ND	ND	7.57	0.28	ND
760	0.2	0.08	ND	ND	ND	ND	ND	0.18	ND
1,050	3.2	0.10	ND	ND	ND	ND	ND	ND	ND
A	2.84	1.71	3.05	0.66	0.67	0.09	1.17	2.14	0.18
B	1.86	0.21	0.66	0.42	0.40	ND	0.26	1.12	ND
C	93	33	70	5	26	2	33	29	—

A, Mean concentrations found in No. 20 net plankton (includes all size fractions); B, calculated mean concentrations for No. 6 net plankton (based on size fractions $> 202 \mu\text{m}$); C, total PCB residues ($\times 10^{-8} \text{ g m}^{-3}$) associated with No. 20 net plankton. All estimates are weighted according to the standing crop of the different size fractions. ND, Not detectable.

The results summarised in Table 1 show that PCB concentrations were inversely related to particle size, and that this trend persisted in spite of a thirty-four-fold difference in residue levels between composite samples (Table 1, A). This finding is consistent with the known affinity of organochlorines for particulate matter⁷ and indicates that the acquisition of contaminants is directly proportional to particle surface area. Indeed, there is a strong linear correlation ($r=0.95$; $P<0.01$) between the surface to volume ratios of the different size fractions (calculated assuming that plankton are spherical with a radius equal to half the mesh size of the screen) and their average PCB content. This relationship, however, provides only a partial explanation, for on three occasions phytoplankton (principally diatoms) from the 102 μm screens had considerably lower residue levels than the 153 μm fractions (mainly small copepod nauplii).

To our knowledge the 73 μm to 202 μm particles contain the highest PCB concentrations ever reported for natural plankton. Other measurements of coastal and oceanic Atlantic zooplankton range from 0.018 to 0.64 p.p.m. wet weight², whereas samples from the Clyde in Scotland and the Stockholm Archipelago were somewhat lower, ranging from about 0.03 to 0.35 p.p.m. wet weight (recalculated from Jensen *et al.*¹).

Part of this apparent discrepancy between our results

conclusion by noting that most of the DDT residues in seawater are probably bound to particles $< 2 \mu\text{m}$ in diameter which, in turn, account for most of the surface area of suspended particulate material.

Estimates of the total mass of PCBs associated with No. 20 net plankton from the Gulf are given in Table 1, C. Although the overall degree of contamination was very variable, ranging from $93 \times 10^{-8} \text{ g PCB m}^{-3}$ on June 3 to $2 \times 10^{-8} \text{ g PCB m}^{-3}$ on August 1, organochlorines did not seem to accumulate during the study period. On the contrary, the obvious irregularity in residue levels suggests that PCBs entered the plankton in discrete pulses.

For the southern Gulf of St Lawrence, atmospheric input is the most likely source of supply as various organochlorines, including PCBs, have been detected in rainwater and aerial fallout^{8,9}. Moreover, the prevailing wind in summer in this region is from the SSW, meaning that the study area is subject to airborne contaminants originating in the heavily populated St Lawrence River valley and east coast of the United States. This interpretation is supported by Fig. 1, which shows a significant linear correlation ($r=0.86$, $P<0.01$) between PCB contamination (Table 1, A) and the cumulative amount of rainfall 10 to 20 d before sampling¹⁰. Residue levels were not correlated with the amount of rainfall occurring 10 d before sampling, indicating that there is a 2 to 3 week lag between the

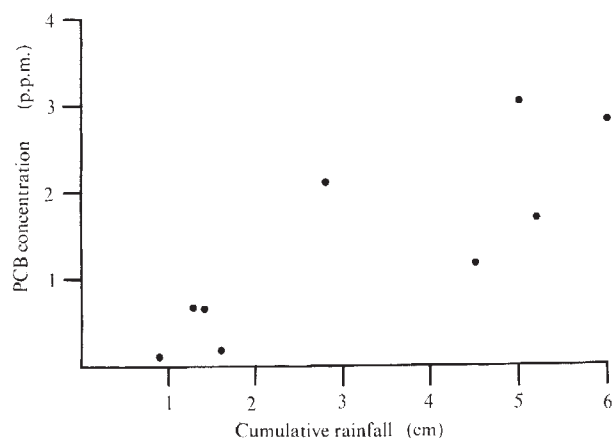


Fig. 1 Relation between the amount of rainfall at Summerside, Prince Edward Island, 10 to 20 d before sampling and average PCB concentrations (p.p.m. wet weight) in No. 20 net plankton.

apparent supply of pollutants and a noticeable uptake by plankton. (The fact that the planktonic community may have been some distance from the station 10 to 20 d before sampling does not affect our argument, as there is an equally good correlation between plankton residues and the average amount of rainfall occurring at Summerside, Prince Edward Island, and a weather station 181 km upstream.)

The evidence presented here indicates that PCBs are transported from the atmosphere to the sea surface, and then on to the plankton. Such a scheme is compatible with current theories^{8,11}, which postulate that organochlorines in surface waters are in equilibrium with those reaching the air-sea interface either adsorbed on particulates or as a vapour, whereas the plankton are assumed to equilibrate with the water-soluble residues. If the atmosphere is indeed the primary source of input to the Gulf, then PCBs will be concentrated near the sea surface after heavy rainfalls. Although the fate of these residues is somewhat uncertain at present, some contaminants are likely to be transported back to the atmosphere by evaporative co-distillation, while the rest would, in time, become dispersed throughout the water column by various biological and physical transport mechanisms.

Cox¹² found that DDT has a half-life of 4 to 10 d in euphausiids. We suggest that PCBs associated with large plankters could be cleared at similar rates, and that this mechanism augmented by a rapid turnover in biomass, especially of small plankters, could account for the low residue concentrations in Gulf plankton following periods of light precipitation. As a general conclusion then, it seems that PCB levels in marine plankton will depend not only on the size composition of the community, but also on spatial and temporal variations in the supply of contaminants.

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Antibody Response to Tapeworm (*Hymenolepis diminuta*) in the Rat

Hymenolepis diminuta, an unarmed, non-invasive tapeworm of the small intestine, is classically regarded as being non-immunogenic in the rat¹. In this host, no pathology is seen², no protective response is stimulated¹, and the host-parasite system appears to be maintained in equilibrium. Hopkins *et al.*³ suggested that transmucosal antigen transport may occur in such host-parasite systems, and produced evidence⁴ that the rejection of *H. diminuta* in mice is mediated immunologically. Here we present direct evidence of an antibody response to a parasite with no invasive phase, producing no pathology, and living in equilibrium in an environment which may be considered as entirely external to host tissue. This model may find application in the wider field of transmucosal antigen transport and presentation, for example as in food and pollen allergies, as well as serving to illustrate the distinction between the production of an immune response and the development of protective immunity in host-parasite systems.

Eighty-five female Sprague-Dawley SPF rats (200–250 g) were infected by stomach tube under light ether anaesthesia with five or twenty-five cysticercoids of *H. diminuta* dissected from the flour beetle *Tribolium confusum*. Groups of four rats taken at weekly intervals, together with uninfected controls, were anaesthetised with carbon dioxide and bled out by cardiac puncture, and the parasites were recovered and counted. Individual sera for each group were pooled and stored at -70°C . The complete experiment was repeated, the two sets of sera examined independently and the data combined to minimise effects of individual variation. Parasite-specific antibody was detected by 4 h and 72 h homologous passive cutaneous anaphylaxis (PCA) and by the indirect fluorescent antibody method. Antigen was prepared by homogenising freeze-dried adult tapeworm tissue in borate-buffered saline (pH 8.6), sonicating at 21 kHz for 5 min at 0°C and centrifuging at 4°C for 20 min at 20,000g. The supernatant was decanted, a sample removed for protein estimation by the method of Lowry, and stored at 4°C with the addition of 1/10,000 merthiolate as preservative. The insoluble residue was fixed in an ascending methanol gradient to 90%, washed twice in saline and freeze-dried in suitable aliquots to be used as a particulate antigen for fluorescent studies.

The indirect fluorescent antibody technique was performed as described by Kane *et al.*⁵, taking the highest serum titre which gave definite fluorescence as the end-point. For PCA studies, immunoglobulins from a 1 ml sample of serum were precipitated with 50% ammonium sulphate, washed twice, redissolved in 1 ml phosphate-buffered saline (pH 7.2) and dialysed exhaustively against the same buffer. The partially purified immunoglobulins were injected intradermally in doses of 0.05 ml or 0.1 ml into male Sprague-Dawley rats (200–250 g), subsequently challenged intravenously 4 h or

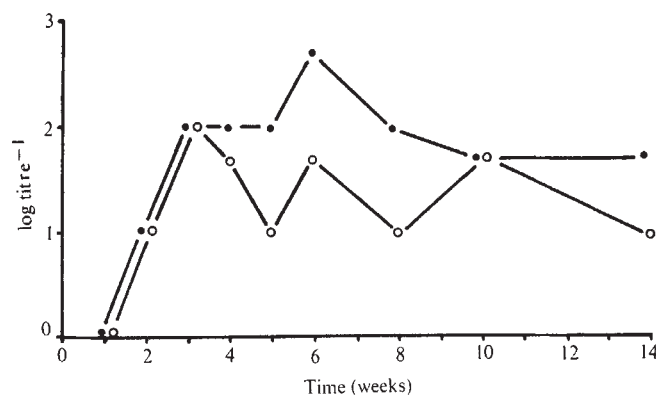


Fig. 1 Evolution of parasite-specific antibody with time, as determined by indirect fluorescence. ●—●, In rats infected with five worms; ○—○, in rats infected with twenty-five worms.

72 h later with 5 mg soluble antigen in 0.5 ml saline together with 1 ml 0.25% Evans blue dye. Reactions were scored 30 min later as previously described⁶.

Table 1 Number of Worms Recovered from Rats Fed Five or Twenty-five Cysticercoids of *H. diminuta*

Age of infection (weeks)	Mean number of worms recovered $\pm$ s.d. 5 Cysticercoids fed	25 Cysticercoids fed
1	4.8 $\pm$ 0.4	24.3 $\pm$ 0.7
2	4.8 $\pm$ 0.4	22.4 $\pm$ 4.0
3	4.5 $\pm$ 0.5	18.8 $\pm$ 4.9
4	4.9 $\pm$ 0.4	16.9 $\pm$ 7.7
5	4.8 $\pm$ 0.7	11.9 $\pm$ 7.1
6	4.3 $\pm$ 0.8	12.1 $\pm$ 7.5
8	4.9 $\pm$ 0.4	12.4 $\pm$ 6.7
10	4.4 $\pm$ 0.8	11.5 $\pm$ 7.5
14	4.8 $\pm$ 0.6	9.5 $\pm$ 11.1

Worm recoveries are summarised in Table 1. Five worm infections were stable, with no evidence of worm loss up to 14 weeks post-infection, confirming the accepted status of *H. diminuta* in the rat. Hosts harbouring twenty-five worm infections, however, showed a progressive loss of worms over the 14 weeks of the experiment. The gradual and incomplete nature of the loss, together with the stability of the low level infection and the similarity of antibody response between the two infection levels, suggests that this loss of worms was not immunologically mediated. The phenomenon may be associated with the 'crowding effect' described by Roberts⁷, and is probably due to simple competition for available resources as the worms increased in size.

The serological data are summarised in Figs 1 and 2. Figure 1 shows the change in parasite-specific fluorescent antibody titre with time for five and twenty-five worm levels.

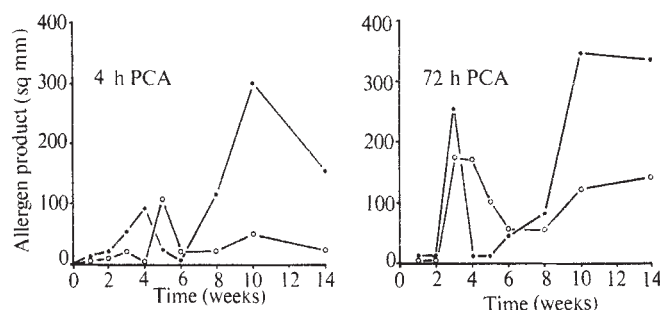


Fig. 2 Evolution with time of parasite-specific antibodies mediating 4 and 72 h PCA. The allergen product was calculated by multiplying the opposing diameters of the reaction at 30 min after challenge together with a subjective 0-4 score for intensity of colour. ●—●, Rats infected with five worms; ○—○, rats infected with twenty-five worms.

Peak titres were reached by week 3, and persisted relatively unchanged through to week 14.

Four hour homologous PCA is thought to be mediated in the rat by a 7S IgG_a antibody, and 72 h homologous PCA by a true reagenic-type antibody apparently analogous to human IgE⁸. Figure 2 summarises the evolution of 4 and 72 h homologous PCA antibody with time; a biphasic type of response is seen for both antibody types and for both levels of infection. The significance of this feature is not known, although it may possibly reflect qualitative and quantitative changes in immunogens present in the gut as the worms become mature. In this respect, it would be interesting to study in more detail the specificity of early and late antibody populations, particularly in relationship to, for example, the breakdown of gravid proglottids with the consequent appearance of eggs free in the gut lumen of the host.

It is unusual to see both 4 and 72 h PCA antibody responses following helminth infection. Generally such infections lead to production of true reagenic-type antibody but no skin-sensitising 7S IgG, whereas immunisation with parasite antigens leads to production of such 7S IgG⁹. The biphasic type response and the production of both types of skin-sensitising antibody clearly merit further study in relation to less stable host-parasite systems and to non-parasitic allergy.

These data unequivocally demonstrate a broad-spectrum antibody response to an entirely non-invasive, non-pathogenic lumen-dwelling parasite. Antigens must cross intact mucous membranes to be presented in immunogenic form for the development of well known food and pollen allergies; the interaction between the rat and *H. diminuta* may be a valuable model both for study of the mechanisms and pathways whereby such transport may occur, and for the emphasis and examination of the functional distinction between protective and non-protective immune responses.

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Ultrasonic Surveillance of Subatmospheric Decompression

DECOMPRESSION sickness is a recognised hazard of unprotected high altitude flight¹. As attempts to detect circulating bubbles in men during decompression from simulated dives using superficial ultrasonic Doppler devices have apparently been successful^{2,3}, we have attempted to detect similar

evidence for bubbles in men undergoing rapid decompression to simulated high altitude.

The one man capsule of a decompression chamber at the Royal Air Force Institute of Aviation Medicine, Farnborough, was used. The ultrasonic equipment was a modified Sonicaid foetal heart detector (operating frequency ~ 2.15 MHz). As for simulated dives³, the transducer was strapped to the chest over the fifth left intercostal space to monitor the ventricles of the heart.

The principle of the ultrasonic Doppler device is well known⁴. Over the heart our instrument gave an audio signal like a heart beat which we recorded.

Abnormal signals associated with hyperbaric decompression have been described as 'plops'². The human ear is still the most sensitive detector for these events. Although the 'plops' appear more clearly when the audio signal has been passed through a 600 to 800 Hz band-pass filter, marginal bubble signals cannot yet be recognised automatically.

Details of the decompressions given in the present series are shown in Table 1. All, except the last, have been used to simulate sudden loss of cabin pressure when training RAF air crew. The most severe pressure ratio is 10.4:1 (run 7). All decompressions were fairly short except the last, which was deliberately extended.

There may well be a critical time as the apparently hazardous procedure known by divers as 'surface decompression'⁷ and by compressed air workers as 'decanting'⁸ is in fact often practised safely⁹.

These procedures are permitted so long as no more than 5 min elapse between leaving pressure and getting to pressure in the recompression chamber^{7,8}. Thus, there may be a 'safe period' of at least 5 min in which significant bubble growth is not expected. As the first eight of our experiments allowed up to 2 min at maximum altitude, giving at most about 6 min above the 18,000 foot threshold, it is possible that no bubbles were detected because insufficient time was allowed. The last run (No. 9) was therefore deliberately extended so that the subject was above the threshold for over 12 min. In spite of exposure to less than 1/5 At for more than 10 min, no bubble signals were heard in the Doppler output either during the decompression or for 5 min thereafter.

We must conclude, therefore, that bubbles will probably be rarely detected in short-term subatmospheric decompressions. This is entirely consistent with the relative incidence of decompression sickness when compared with that found for hyperbaric decompression¹⁰.

We thank the Director General of RAF Medical Services

Table 1 Simulated Decompression of Experienced Personnel Monitored with Superficial Ultrasonic Doppler Devices

Run No.	Subject	Date	Base altitude ($\times 1,000$ feet)	Ascent time (s)	Final altitude ($\times 1,000$ feet)	Period at final altitude including ascent (min)	Symptoms reported	Abnormal Doppler signals
1	PP	February 6, 1973	8	12	40	1	Mild gut pain	None
2	JM	February 6, 1973	8	12	40	2	Mild gut pain	None
3	CW	February 6, 1973	25	3	54	1	None	None
4	AM	February 6, 1973	25	3	54	1	Moderate gut pain	None
5	BP	February 6, 1973	8	30	40	2	Mild gut pain	See text
6	BP	February 6, 1973	8	30	40	1		See text
7	SL	February 6, 1973	8	30	40	2	Toothache	None
8	AM	February 7, 1973	8	12	60	1	Tingling left hand	See text
9	DD	February 7, 1973	8	12	40	10	Sleepy	None

Standard oxygen equipment and, where required, pressure breathing suits were used. Throughout each run ECG and chamber pressure were recorded together, where appropriate, with mask oxygen flow and mask pressure.

All subjects were experienced and able to report any relevant symptoms. The full Doppler signal was recorded throughout each run, which consisted of a 2 min control period at ground level, a gradual ascent to base altitude, a rapid ascent followed by a period at maximum altitude, a gradual descent and a further period of 5 min at atmospheric pressure.

No abnormalities were found in the ECG recordings and no symptoms of decompression sickness were reported with the exception of transient urticaria and petechiae on one run (No. 8). No clear bubble signals were heard. Occasional indistinct abnormal signals were detected in three of the runs (Nos 5, 6 and 8). These did not occur at maximum altitude, but during the recompression. The other runs gave clear heart signals throughout.

The pressure equivalent of a height of 18,000 feet is generally regarded as the safe limit for subatmospheric decompression¹. This corresponded to Haldane's rule⁵ for hyperbaric decompression that the absolute pressure can be halved with safety. In modern diving practice a smaller ratio is used⁶ and we have detected many circulating bubbles with the Doppler apparatus after a simulated decompression of ratio 1.73:1 (ref. 3).

In the present series, no clear evidence for bubbles was found even for subatmospheric pressure ratios of over 10:1. One reason for this may be that the time at altitude was insufficient for bubbles to grow to detectable size.

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Isolation of a New Cardiotoxic Protein from the Edible Mushroom, *Volvariella volvacea*

THE cyclic toxic peptides, phalloidins and amanitins, have been isolated from *Amanita phalloides*; they caused violent emesis and diarrhoea or even death 10 to 24 h after ingestion^{1,2}. Their chemical structures have been elucidated by Wieland³, and the action of the amanitins was found to be the inhibition of RNA polymerase⁴.

We have isolated a new toxic protein from the edible mushroom, *Volvariella volvacea*, which is widely eaten in the Orient and is canned for local consumption and export. From spring to autumn *Volvariella volvacea* forms basidiocarps which appear as ovate bodies with an egg-shaped greyish brown cap that becomes darker at the top. Within 1–2 d, the cap opens, breaking the universal veil, and reaches a diameter of up to 15 cm. The surface of the cap is brown and fibrous, and patterned with darker, radially orientated filamentous streaks. The lamellae are pink, and the stem is surrounded by a dark brown volva. The mushroom has neither smell nor taste.

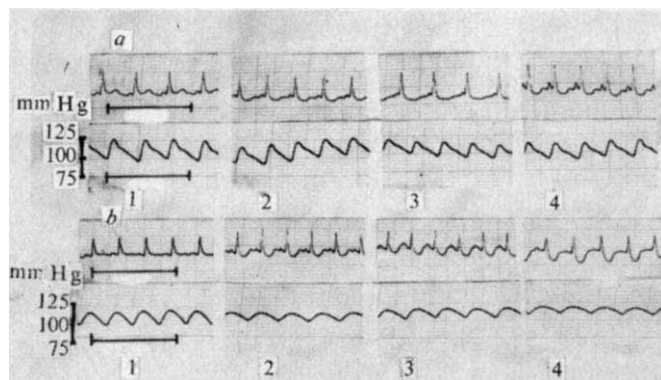


Fig. 1 Effects of volvatxin A on the electrocardiogram and arterial blood pressure of a 2 kg cat, pentobarbital anaesthetised (30 mg per kg body weight intraperitoneally). Upper trace, ECG (lead II); lower tracing, arterial blood pressure. *a*, Injection of 0.7 mg per kg body weight volvatxin A; 1, 0 time; 2, 5 min; 3, 15 min; 4, 75 min. *b*, Injection of 1 mg per kg body weight volvatxin A; 1, 0 time; 2, 3 min; 3, 5 min; 4, 7 min. Bar represents 1 s.

We name the toxic protein obtained from *Volvariella volvacea*, volvatxin. This is extracted from the basidiocarps with cold, 0.8 N acetic acid, 0.016 M mercaptoethanol and 0.001 M EDTA by homogenising in a Waring blender. The proteins that were precipitated between 0 and 50% saturation of ammonium sulphate were collected. After removing the salt by dialysis against 0.016 M mercaptoethanol and 0.001 M EDTA at 4° C for 36 h, the dialysed solution was applied to a DEAE-Sephadex 50A column (5×50 cm) and eluted with 0.1 N acetic acid and 0.016 M mercaptoethanol.

Only one major protein peak was obtained, which was pooled and named volvatxin A. Its toxicity was measured by intraperitoneal injection of various amounts of the toxic protein into male NMRI strain mice, weighing 20 ± 1 g. The LD₅₀ was found to be 1.23 mg kg^{-1} body weight. Volvatxin A was found to have three major biological activities. The first was a direct haemolytic action: it can completely lyse human group O red blood cells at a concentration of $2 \mu\text{g ml}^{-1}$. No phospholipase A activity was detected in volvatxin A. Second, it caused a writhing reaction when administered intraperitoneally. The time of onset was 4 min and a dose of 2–5 mg per kg body weight caused about thirty-six writhing reactions within 10 min of the first writhing reaction. Third, in isolated toad hearts the toxic protein caused ventricular systolic arrest

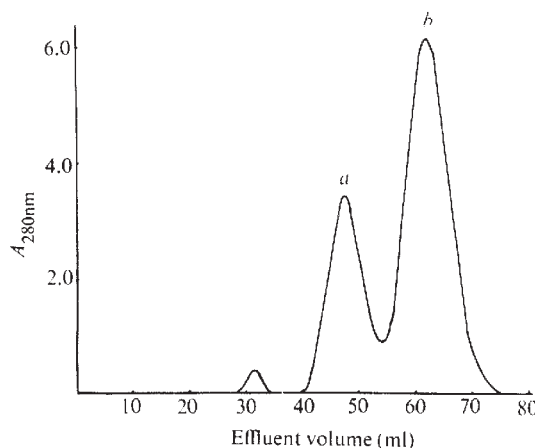


Fig. 2 Chromatogram illustrating the homogeneity of volvatxin A on a column of Sephadex G-75 equilibrated with pH 7.0, 0.01 M phosphate buffered normal saline.

at a dose of 0.1 mg ml^{-1} ; in cats an intravenous injection of the protein produced changes in ECG at a dose of 0.7 mg per kg body weight: it depressed the ST segment and inverted the T wave, but no significant changes in blood pressure were noted. The abnormal ECG did not recover until 75 min after the injection (Fig. 1*a*, 2–4). With 1 mg per kg body weight volvatxin A the changes of ECG were much more pronounced and irreversible. A complete atrio-ventricular block with aberrant QRS-T complex and nodal rhythm were observed within 5–7 min after the injection, though the arterial pressure was still normal (Fig. 1*b*, 2–4). The toxicity and biological activities of the toxic protein were completely eliminated by heating at 100° C for 20 min.

The homogeneity of the volvatxin A was tested by gel filtration and the results are shown in Fig. 2. There are

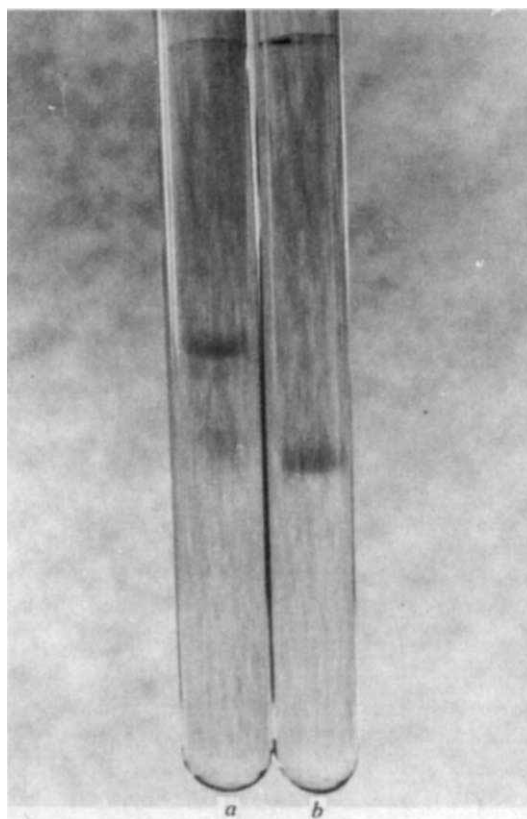


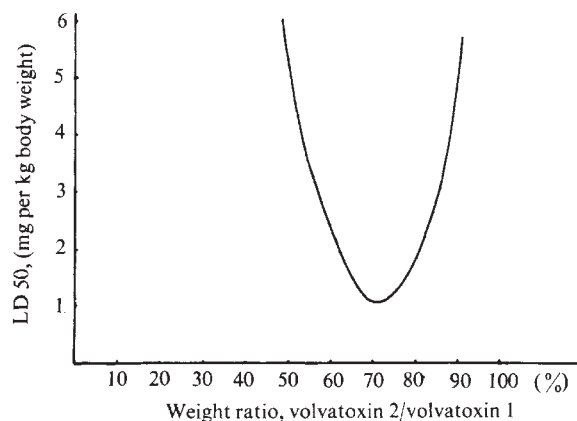
Fig. 3 Sodium dodecyl sulphate gel electrophoresis of volvatxin A1 and A2. The gel on the left represents volvatxin A1.

Table 1 Amino Acid Composition Analysis of Volvatoxin A1 and Volvatoxin A2

Amino acid	Volvatoxin 1 residues per 12,500	Volvatoxin 2 residues per 12,500
Lys	9	10
His	1	1
Arg	1	1
Asx	12	11
Thr	10	6
Ser	8	9
Glx	10	11
Pro	3	3
Gly	7	5
Ala	7	8
Cys	—	1
Val	10	6
Met	1	1
Ile	5	5
Leu	9	9
Tyr	5	4
Phe	7	8
Try	5	5
Total	110	104

two major components in volvatoxin A, volvatoxin A1 and volvatoxin A2. The molecular weights of volvatoxin A1 and A2 were found to be 50,000 and 24,000, respectively, by gel filtration⁵ and SDS acrylamide gel electrophoresis⁶. Both proteins are homogeneous with respect to gel filtration and SDS acrylamide gel electrophoresis (Fig. 3). The possibility that volvatoxin A1 is an aggregation product of volvatoxin A2 is excluded by amino acid composition analysis (Table 1) and tryptic map analysis (Fig. 4).

The LD₅₀ of volvatoxin A1 and A2 was determined by intraperitoneal injection and it was found that both proteins lost their original toxicity when they were tested separately and when the LD₅₀ of volvatoxin A1 and A2 was higher than 40 mg per kg body weight. When the two proteins were

**Fig. 5** Toxicity of the mixture of volvatoxin A1 and A2.

mixed together, however, the toxicity of the mixture increased (Fig. 5). The optimal ratio is 1:3 which is the ratio of the two proteins existing in volvatoxin A, and the LD₅₀ of the mixture at the optimal ratio is almost the same as that of volvatoxin A.

The biological activities of the two isolated proteins were investigated. Volvatoxin A2 carries all the haemolytic activity of the volvatoxin A. The minimum concentration for complete lysis is $1.6 \mu\text{g ml}^{-1}$. So far no significant biological activity has been detected in the volvatoxin A1.

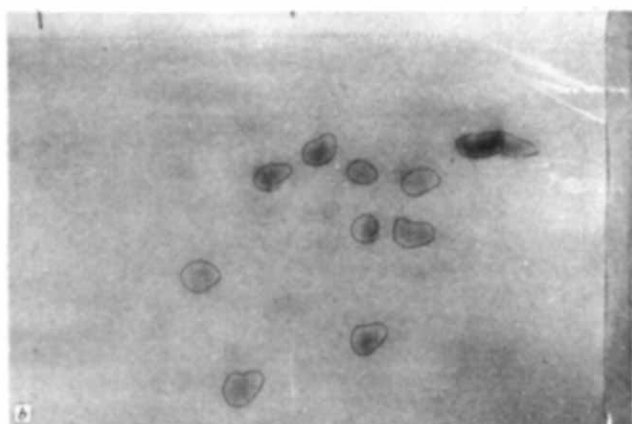
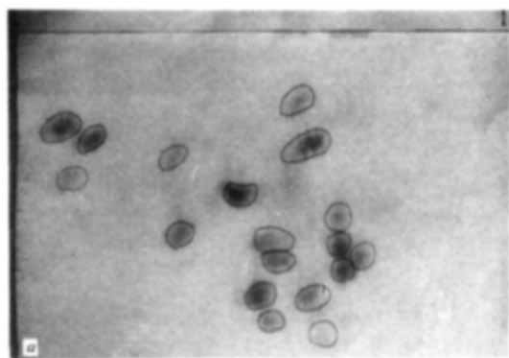
These findings are very similar to those of Hendon and Fraenkel-Conrat⁸, who showed that crotoxin is composed of two kinds of protein, one acidic and one basic. The acidic protein lacks the haemolytic and neurotoxic activity of crotoxin and the basic protein shows only the high, indirect haemolytic activity; a mixture of the two components shows the high neurotoxicity of crotoxin.

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**Fig. 4** Tryptic peptide maps of volvatoxin A1 and A2. Upper map, volvatoxin A2.

Loss of Bacterial Motility under Pressure

BACTERIAL motility is thought to be ecologically important, especially in environments containing inadequate nutrients. Many bacteria have highly specialised tactic systems that allow them to detect physical or chemical gradients and to swim either to or away from the source. Motility and chemotaxis seem to be important also in bacterial film formation on surfaces and in marine fouling¹.

Microorganisms in the ocean or other bodies of water may be affected significantly by hydrostatic pressure, which increases by about one atmosphere (atm) for every 10 m depth. As

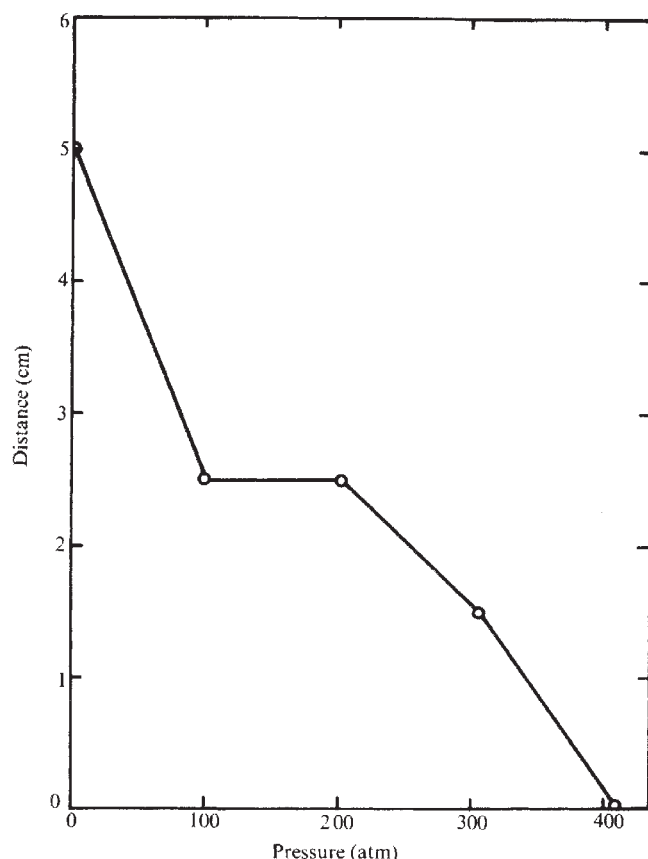


Fig. 1 The effect of pressure on motility of flagellate *E. coli* B275. Capillary tubes were prepared as described in the text, and the position of the moving frontier of bacteria was determined after 75 min at 24° C at the indicated pressures. The medium through which the bacteria moved contained L-serine as energy source but would not support growth of the auxotrophic organism used because it lacked required amino acids. The points shown represent average results of two separate experiments; they indicate corrected values with a 2-cm subtraction for tubes at 1 atm and a 3-cm subtraction for pressurised tubes.

Table 1 Bacterial Motility under Pressure

Organism	Motility at				
	1 atm	102 atm	204 atm	306 atm	408 atm
<i>Escherichia coli</i>	+++	+++	++	—	—
<i>Serratia marcinobura</i>	+++	+++	+	—	—
<i>Proteus mirabilis</i>	+++	+++	+	—	—
<i>Salmonella typhosa</i>	+++	++	+	—	—
<i>Bacillus licheniformis</i>	+++	++	—	—	—

Bacteria were inoculated into tubes of soft agar medium by stabbing to a depth of 1.4 cm with marked needles. The medium used for the Gram-negative bacteria was prepared by dissolving 8 g Difco nutrient broth, 25 g Difco peptone and 3 g agar in 1 l of deionised water. The medium for *B. licheniformis* was prepared by dissolving 0.2 g maltose, 0.2 g Difco peptone, 0.4 g Difco yeast extract and 0.3 g agar in 100 ml deionised water. The pH of the solution was adjusted to 7.5, the solution was autoclaved, and then 1.0 ml of 1 M potassium phosphate buffer (pH 7.0) was added. After each tube was inoculated, 1 ml of sterile 2% agar was poured on top to seal the inoculation site. The space above the agar was filled with sterile water, and the tubes were sealed with serum caps before pressurisation. All cultures were incubated at room temperature until growth was clearly apparent in both pressurised and unpressurised tubes—48 h for the Gram-negative bacteria and 72 h for *B. licheniformis*.

vitamin-free casein hydrolysate replaced the amino acid mixture.

For frontier assays, each tube was marked off into eight compartments 1 cm long and broken at the liquid-agar interface at end B. The contents of each compartment were removed with a very fine tip pipette and used to inoculate a tube containing 1 ml of eosin-methylene-blue broth plus 100 µg streptomycin per ml. Tubes were incubated for at least 48 h at 30° C to determine whether or not bacteria had migrated into each compartment.

Figure 1 presents data on the effect of pressure on movement of bacteria in capillary tubes during 75 min of incubation. At 1 atm, the advancing front of bacteria travelled nearly the entire length of the tube; on the other hand, at 408 atm there was no detectable movement. Motility was inhibited at 102 atm, even though growth is unaffected by this low pressure.

early as 1891, Regnard² reported that pressure inhibits bacterial movement. Subsequent studies³ confirmed and extended Regnard's observations but left the nature of the inhibition undefined. Our recent observations indicate that pressure inhibits both the formation of new flagella and the functioning of previously formed ones.

To assess qualitatively the effects of pressure on motility, we used a soft agar medium and observed growth and movement away from the line of inoculation. Typical results for several bacteria are presented in Table 1. Movement was progressively restricted with increasing pressure, and no bacterium was motile at 306 or 408 atm, even after prolonged incubation, although all of them grew at these pressures.

For more detailed studies we used a streptomycin resistant *Escherichia coli* strain (B275), obtained from Dr J. Adler of the University of Wisconsin, and melting point capillary tubes. A mark was made on each tube 2 cm from one end (end A). The tube was filled from the other end (B) with medium up to the mark. Then end B was plunged into 2% agar gel until the medium moved to within 1 mm of end A. Washed cells were introduced into end A with a finely drawn out Pasteur pipette, and end A was plunged into agar until a 1.5 cm plug was introduced. Finally, the ends of the capillary tubes were sealed with a 1:1 mixture of wax and Vaseline before being placed in test tubes with end A down. The test tubes were filled with sterile water, sealed with serum caps, placed in pressure cylinders, pressurised with a hydraulic pump and laid on their sides. Media used for growth and for motility assays were those described by Adler and Dahl⁴, except that

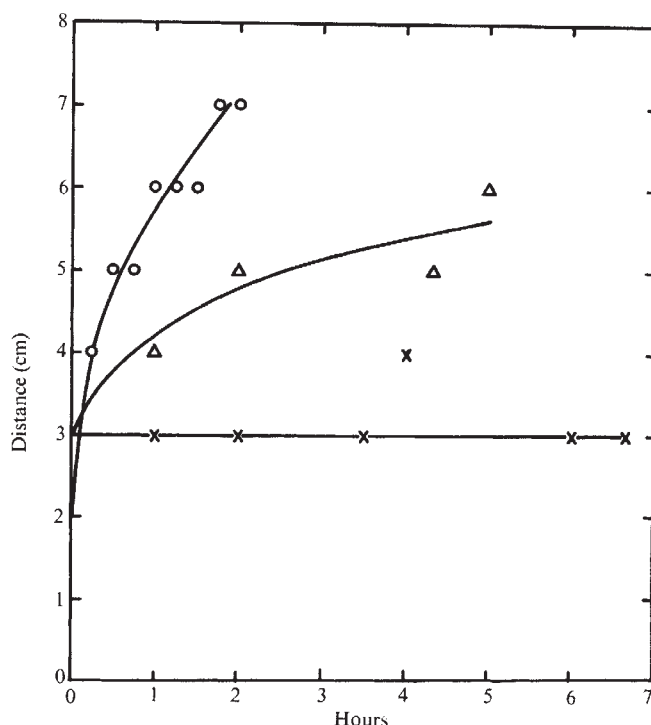


Fig. 2 Time course of movement of *E. coli* B275 along capillary tubes at 24° C and 1 atm (O), 408 atm (Δ) and 612 atm (x) pressure.

Slowing of movement could not be related to increased medium viscosity, which changed less than 1% under pressure⁵.

The points of Fig. 1 were used for a least squares approximation of a linear relationship between distance travelled and pressure. The analysis indicated that the rate of movement decreased by 1.08 cm per 75 min per 100 atm increase in pressure. The apparent activation volume for the inhibitory reaction is equal to $2.30 (d \log k/dP)RT$, where k is the rate, P the pressure, R the gas constant and T the Kelvin temperature. The calculated value of 66.9 ml mol^{-1} is sufficiently large to suggest that inhibition involves some sort of cooperative phenomenon.

Figure 2 presents data from a 7 h experiment. At 1 atm the advancing front of bacteria reached the end of the capillary tube in about 2 h, but at 408 atm the bacteria took about 5 h to travel the distance travelled in 75 min at 1 atm. Movement was stopped completely at 612 atm. The first two compartments of 1-atm tubes always contained bacteria, whereas the first three compartments of pressurised tubes always contained bacteria, even at 612 atm. This difference in zero value is related to pressurisation-depressurisation times and is reflected in the intercepts of Fig. 2.

Although *E. coli* cells were not motile at 612 atm, they retained flagella as indicated by their appearance in smears stained with Leifson's flagellar stain⁶. Gerber and Noguchi⁷ found that *in vitro* aggregation of flagellin to form filaments results in a dilatation of 157 ml per ml of flagellin, and so should be inhibited by pressure. Our results indicate that pressure does not cause disaggregation of flagella *in vivo*.

To see if bacteria can form flagella under pressure, we stained cells grown at various pressures. Inoculum bacteria shown in Fig. 3a were almost devoid of flagella. They were obtained from cultures grown at 37° C in glucose-minimal medium. Glucose acts as a repressor of flagella synthesis⁸, but repression can be relieved by adding cyclic AMP to the cultures⁹. The inoculum bacteria were washed and added to casein hydrolysate medium plus FC-80 (3M Co., St Paul, Minnesota) for growth at 1, 102, 204, 306 and 408 atm. FC-80

is a water-immiscible fluorocarbon which acts as a reservoir for dissolved oxygen to supply cultures in enclosed vessels. As Fig. 3b shows, cells grown at 1 atm synthesised many flagella, those grown at 102 atm synthesised few flagella (Fig. 3c) and those grown at 204, 306 or 408 atm (Fig. 3d, e and f) were nonflagellate. Clearly, flagella synthesis is much more sensitive to pressure than is growth or protein synthesis. Landau^{10,11} found that 272 atm stimulated labelled amino-acid incorporation by this bacterium, 408 atm had no effect, and higher pressures were inhibitory. He also found¹² that enzyme induction was very pressure sensitive and that 265 atm nearly completely inhibited induction of the *lac* operon.

Flagella formation is a complex process involving assembly as well as synthesis of proteins. Perhaps assembly is more sensitive to pressure than is synthesis. Also, it seemed possible that pressure might block induction of synthesis, but we were unable to reverse the inhibitory effect of pressure by adding 5 mM cyclic AMP to cultures.

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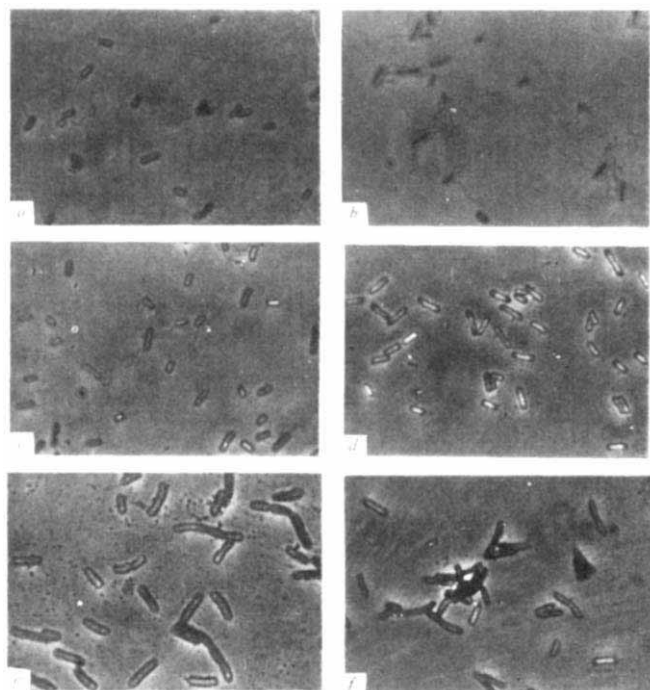


Fig. 3 Effect of hydrostatic pressure on flagellation of *E. coli* B275. Inoculum cells (a) were grown in glucose-minimal medium at 37° C and were subcultured into casein hydrolysate medium for growth at 1 (b), 102 (c), 204 (d), 306 (e) and 408 (f) atm.

Production of Calcite (Calcium Carbonate) Crystals by Soil Bacteria is a General Phenomenon

CERTAIN bacteria form crystals from the solutes in their aqueous environment, and some authors have associated this activity with the extensive deposits of CaCO_3 in such places as the Grand Bahama, in spite of the belief that physico-chemical effects, such as rapid changes in pH, salinity and temperature, are responsible¹⁻⁴. Drew⁵ isolated a denitrifying bacterium able to form CaCO_3 crystals in liquid media and named it "*Bacterium calcis*" (later named *Pseudomonas calcis*⁶). Greenfield⁷ obtained aragonite (another form of CaCO_3) crystals in cultures of *Pseudomonas* in an artificial seawater medium containing Na_2CO_3 or $(\text{NH}_4)_2\text{CO}_3$. Buck and Greenfield reported the same result with marine yeast, and claimed that calcium crystals resulted from the accumulation of calcium deposits on the surface of the cells⁸. McCallum and Guhathakurta⁹ observed calcium carbonate deposition by marine bacteria isolated from Bahama Island sediments, and when these were cultured in different media aragonite crystals formed. Shinano¹⁰ described many marine bacteria able to form crystals in liquid media. Ramos-Cormenzana¹¹ has also reported crystal formation by soil

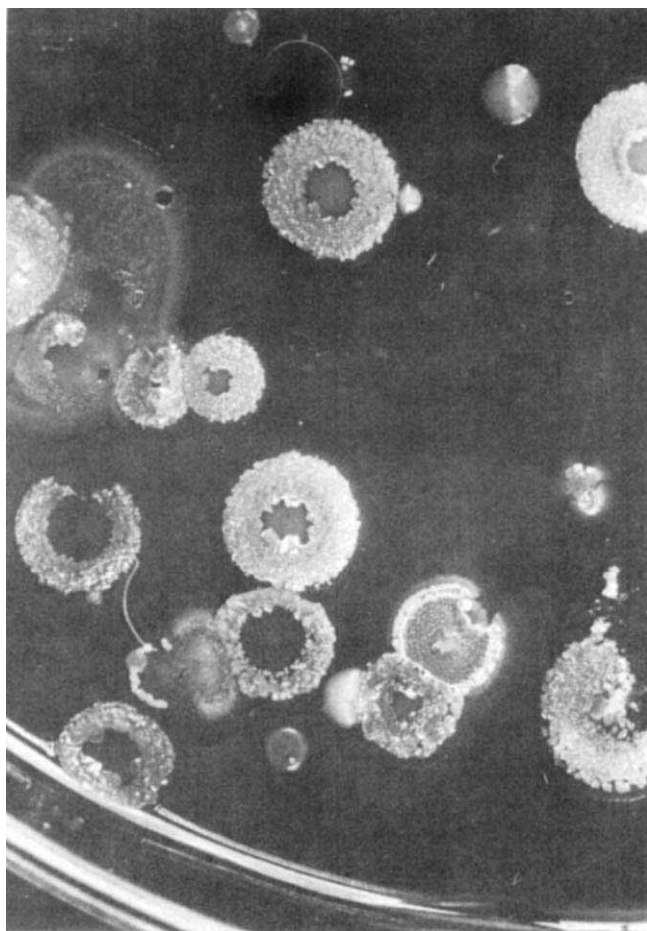


Fig. 1 Macroscopic observations of crystal formation in bacteria. Mixed colonies on plates of B-4 medium after 18 d incubation; colonies which appear free of crystals reveal copious crystalline deposits when examined microscopically.

bacteria in solid media. This, together with the knowledge that previous research concerned only marine bacteria in liquid media, stimulated us to investigate crystal formation by soil bacteria cultured on solid media.

We obtained the best results with B-4 medium, which contains 0.25 g calcium acetate, 0.4 g yeast extract, 1.5 g agar, 1.0 g glucose and 100 ml distilled water, with pH adjusted to 8.0 using NaOH. The same medium without glucose gave good growth and crystal formation, but added glucose speeded the processes. In each case we used uninoculated plates as controls. The appearance of crystals as the bacteria grew could be assessed macroscopically and microscopically. Figure 1 shows several colonies of crystal-forming organisms growing on B-4 medium after 18 d of incubation, and Fig. 2 shows the accumulation of crystals within the colony. The crystals could be scraped off the bacterial growth and washed with water to remove all traces of cells and adhering medium; since they were insoluble in water, they could be washed repeatedly and freed from chemical contaminants.

The crystals were identified physico-chemically by their solubility in HCl, by the Meingen reaction, by infrared spectroscopy, and by petrographic microscopy. The results showed that the crystals were calcite, and X-ray diffraction and comparison with known calcite crystals provided confirmation.

We have isolated 210 organisms able to form crystals on plates of B-4 medium, and found that all the bacteria we isolated from soil, which could grow on B-4, could form crystals. All organisms we have tested from our laboratory collection also formed crystals. These include *Salmonella*

spp., *Azotobacter* spp., *Bacillus pumilus*, *B. subtilis*, *B. megaterium*, *B. cereus*, *B. bulgaricus*, *B. globigii*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Serratia* spp. (non-pigmenting strain), *Citrobacter* spp., *Enterobacter aerogenes*, *E. faecalis* and *Staphylococcus aureus*. They all formed crystals on B-4 agar in 1 to 20 d of incubation. From this we infer that crystal formation is a common phenomenon and that its occurrence is simply a function of the composition of the medium used.

We describe this finding in view of recent reports^{4,10} that only certain bacteria can form carbonate crystals in their growth medium. Shinano¹⁰ isolated 653 cultures of crystal-forming bacteria out of some 5,000 isolates examined. He indicated that no aerobic, Gram-positive, rod-shaped bacteria formed crystals, but we have shown extensive crystal formation by *B. pumilus*, *B. subtilis*, *B. megaterium*, *B. cereus*, *B. bulgaricus* and *B. globigii*.

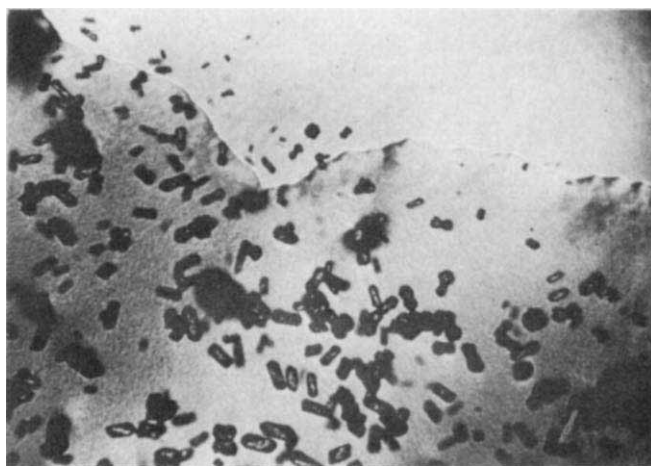


Fig. 2 Microscopic observation of crystal formation by *Proteus vulgaris* (5-d-old culture) in B-4 medium ($\times 27$). The lower part of the photograph shows the cell colony and included crystals, and the top area shows crystals in the medium.

In view of these findings we feel that crystal formation is a function of the medium used and that under suitable conditions most bacteria can form calcite crystals.

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Origin of Chromosome Number Variation in Cultured Plant Cells

CULTURED cells of both animal and plant tissues are characterised by instability of chromosome number and structure^{1,2}. Although there is evidence³ that polyploid plant cell lines may arise from endoreduplicated nuclei in the original explant, the range of chromosome number and structure observed in established cultures strongly points to the origin of these changes during culture. Since the precise distribution of chromosomes at mitotic anaphase is the essential prerequisite for chromosome number stability⁴, this seems the most likely point at which instability could be induced in culture. It has been shown^{6,7} that, in culture, animal cells of various species are characterised by the presence of multipolar mitoses, a feature which would produce daughter nuclei with aneuploid chromosome numbers. Such multipolar mitoses have been briefly noted for plant tissue cultures^{8,9} but their frequencies have not been quantified.

In the present study, the frequencies of bridges, lagging chromosomes and multipolar separations at anaphase and telophase were measured in two suspension culture lines and in seedling root-tip meristems of *Daucus carota* ($2n=18$). The culture lines, originally derived from seedling root segments, and the germinated seedlings, were all of a nucleus stock of *D. carota* donated by Elsoms Ltd (Spalding, Lincs.). Culture line *a* had been grown for thirty-five passages and line *b* for seven passages, each of 18 d duration. Both lines contained 90–95% diploid cells and the remainder tetraploid. The diploid cells of line *a* differed from the normal karyotype by a translocation reducing the chromosome number to 17. Both cultures were maintained on liquid Murashige and Skoog's medium containing 0.1 mg l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) (ref. 10). Such cells were compared with cells from the same initial inoculum subcultured twice on this medium lacking 2,4-D, a treatment which induces embryogenesis^{10,11}. For cytological examination, cells were fixed in 50% (v/v) aqueous formic acid 6 d after transfer to fresh medium, when maximum growth rate and mitotic index were achieved. Root tips, obtained by germination of seeds on moist filter paper, were fixed in Carnoy's solution.

Feulgen-orcein squash preparations of the cultures were analysed for the frequencies of bridges, lagging chromosomes and multipolar configurations at anaphase or telophase. Five slides of each line or treatment were coded and randomised and a total of 200 anaphases plus telophases scored on each slide. A total of 1,000 anaphases plus telophases were scored separately from nine root tip meristems.

As the histogram (Fig. 1) shows, the undifferentiated suspensions of both culture lines showed a significantly ($P<0.05$) higher frequency of multipolar separations than the suspensions initiating embryos and these configurations were absent from the root meristems. The frequency of lagging chromosomes shows a similar trend, though in this respect the root meristem and embryogenic cultures do not differ significantly. In culture line *a*, but not in line *b*, the frequency of bridges was significantly higher than in the root meristems and this probably reflects the presence in line *a* of a population of cells normally containing a dicentric chromosome^{1,9}.

It may be concluded that normal cultural conditions tend to promote abnormalities of the mitotic spindle as evidenced

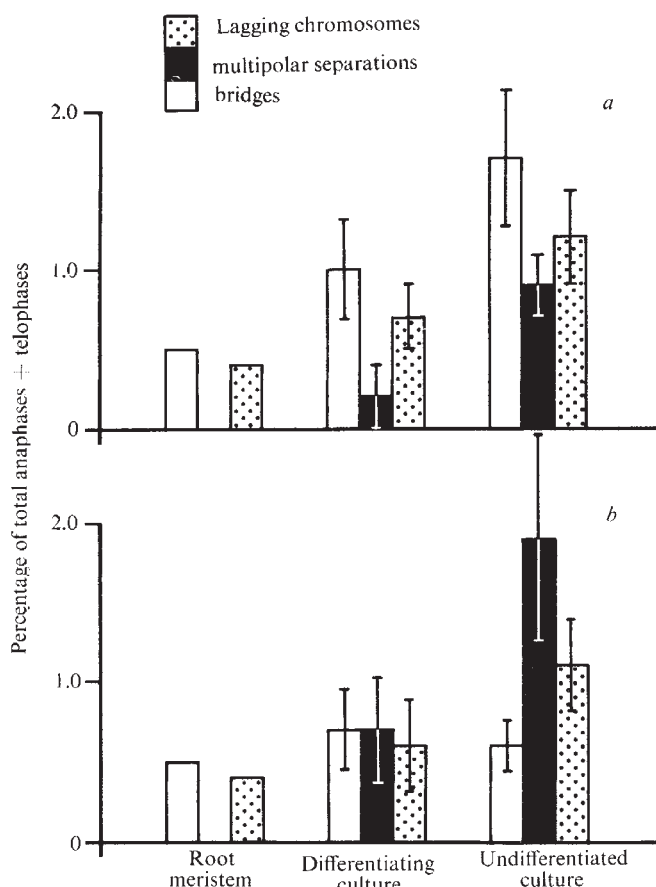


Fig. 1 Frequencies of mitotic abnormalities in two culture lines of *D. carota*, line *a* in its thirty-fifth culture passage, line *b* in its seventh passage.

by the increased frequencies of multipolar separations and lagging chromosomes. It did not prove possible to reliably identify 'C-mitotic' configurations resulting from complete spindle failure, but the partial spindle failure evidenced by multipolar configurations and lagging chromosomes, plus the presence of octoploid and higher chromosome numbers in some carrot cultures, suggests that polyploidisation by spindle failure may occur.

The induction of differentiation in culture seems to reduce the incidence of mitotic abnormalities towards that found in the root tip meristems. In culture, normal mitotic spindle activity could be affected either by the lack of physical organisation of the tissue or by some chemical constituent of the medium. It is, however, not possible here to separate the effects of the increased organisation caused by embryogenesis from the absence of 2,4-D, a substance which at high concentrations is known to induce spindle abnormality^{12,13}. Further, *D. carota* suspensions will not differentiate in the presence of 2,4-D and no diploid line is available which is not embryogenic in the absence of 2,4-D so that suitable controls to distinguish between the effects of 2,4-D and differentiation are not currently possible.

The results demonstrate that mitotic abnormalities likely to give rise to aneuploid chromosome numbers are a significant feature of *D. carota* cultures and though providing some measure of the rate of production of such aneuploid cells, their final frequency will depend largely on the selective effects of the culture system. The frequencies of mitotic abnormalities can, however, be used to test the various physical and chemical components of the culture which may be responsible for induction of cytological variation, a feature which must be controlled if plant tissue cultures are to be fully exploited in plant breeding programmes.

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Appearance of *Hipparion* in the Tertiary of the Siwalik Hills of North India, Kashmir and Pakistan

SIMONS *et al.*¹ have discussed the earliest occurrence of *Hipparion*, a tridactyl fossil equid, in the Tertiary of the Siwalik Hills (Pakistan and India). Their statements are confusing and they have misinterpreted one of my publications. Simons *et al.*¹ reject a possible Chinji occurrence of the genus, partly because "Hussain has pointed out that regardless of the validity of occurrence of the *Hipparion* reputed to have been found near the base of the Chinji, the teeth of these specimens are very large. It is implausible that such animals should have preceded in time the small and structurally different species *Hipparion nagriensis* Hussain".

This is a misinterpretation of my work², in which I clearly stated that the presumed Chinji *Hipparion* teeth cannot be morphologically distinguished from those of the Nagri and Dhok Pathan. *H. nagriensis* from the Nagri Formation is no more primitive than the two Dhok Pathan species (*H. theobaldi* and *H. antilopinum*) in dental pattern or size, but it does differ in cheek tooth hypsodonty and especially in certain functionally important features in the structure of the foot. These characters serve to distinguish *H. nagriensis* from *H. theobaldi* of the Dhok Pathan, which Colbert³ has considered to be present from the lower Chinji through the Dhok Pathan Formations. Simons *et al.*¹ have attributed the same stratigraphical range to *H. antilopinum*, which has never been reported except from the Dhok Pathan Formation; they have thus confused this species with *H. theobaldi* and further clouded an already over-complicated issue. In fact, both of these more advanced Siwalik hipparions (*H. theobaldi* and *H. antilopinum*) are on present evidence restricted to the Dhok Pathan Formation. Neither can be said to be more primitive than the other, but they are separated by size differences^{2,3}, and both seem to be derived *in situ* from *H. nagriensis*².

The presence of *Hipparion* in the Chinji Formation is still uncertain. Representatives of the genus may have entered the sub-Himalayan region in latest Chinji or earliest Nagri times. Definite occurrence of *Hipparion* in the Nagri is the most distinctive faunal feature of this time as compared with the preceding Chinji. Similarly, the first local presence of *Hipparion* in the Vallesian of Spain is the most distinctive feature by comparison to the preceding Vindobonian. In the next younger Dhok Pathan Formation, two different species of *Hipparion* are found which have more

hypsodont teeth and more advanced foot structure than the Nagri *Hipparion*². Similarly, several Turolian *Hipparion* species in Europe are more advanced than the typical Vallesian species (*H. catalaunicum*). On this basis, it is possible to suggest tentatively that the base of the Nagri is correlative with the beginning of the European Vallesian and a similar correlation of the Dhok Pathan and the Turolian is here suggested. Such correlations, however, should be confirmed by other evidence such as entire fauna, flora and absolute dates.

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GENERAL

What Children Do in Spite of Adults' Hypotheses

Bryant and Trabasso¹ suggested that Piaget was wrong when he claimed that children below about 6 yr of age are incapable of making transitive inferences of quantity.

They proposed that the difficulty encountered by the children Piaget tested was not one of transitivity but one of memory, and that therefore it was sufficient to overcome this problem with a modified technique to show up the idea of transitivity in young children.

Bryant and Trabasso's attack focused on this summary of Piaget's view of transitivity: "Piaget and his colleagues propose that the young child cannot infer, for example, that $A > C$ from the information that $A > B$ and $B > C$. . . the child is unable to coordinate the first two, separate items of information in order to reach the correct inferential conclusion about A and C".

If we are interpreting Bryant and Trabasso correctly, they are making an important and legitimate point in emphasising a more basic aspect of transitivity than Piaget, but we feel that their definition is rather different from Piaget's, and that therefore they cannot be justified in claiming that Piaget was "wrong".

Piaget's "definition" of transitivity²⁻⁵ is linked with the whole structure of his system, and in particular with the idea that, for a judgment to be truly an inference, it must be "operational". For this reason, when Piaget studies transitivity using the seriation task for example, the child is only considered to "succeed" if he can "anticipate" the correct actions; if he has a programme or method to proceed, and thus shows no hesitations; or if he is able to perform modifications of the original task (such as insert a new stick into the already assembled sequence) to show that he has understood the ideas involved.

Thus, by concentrating only on the idea that transitivity consists of the ability to combine two asymmetrical relations to deduce a third, Bryant and Trabasso are discarding the Piagetian concept in which, for a behaviour to be a true inference, the child must be able to make use of it in a constructive and coordinated manner. Their view seems to

be that the mere fact of combining the two pieces of information $A > B$, $B > C$ into a third, $A > C$, constitutes an inference. It follows that any test of this elemental kind of transitive inference must be designed in as direct a way as possible, to avoid factors which in a practical situation might prevent the child displaying his ability. Piaget's insistence on "operationality" makes his experiments indirect and complicated, and so his experiments may lead children to fail even though they possess Bryant and Trabasso's elemental transitivity idea.

But just as it is necessary to minimise factors which might lead a child to fail to make an inference he is in fact capable of making, it is also necessary to minimise the possibility that he accomplishes the task in question using extraneous spatial or perceptual clues not requiring an inference to be made at all. Thus, as Bryant and Trabasso point out, it is insufficient to test for the notion of transitivity using a seriation task in which there are only three rods, as there is a simple labelling strategy which permits a solution without requiring the notion of transitivity: A is bigger than B, therefore A is big, B is bigger than C, therefore B is big and C is small. But B cannot be both big and small, so it is neither. Another example of an alternative strategy not requiring the notion of transitivity is the idea, also put forward by Bryant and Trabasso, that if the children are shown and remember the actual sizes of each individual rod, they will have no trouble deciding on the pairs which they have not seen.

The existence of this fairly obvious alternative strategy means that Bryant and Trabasso's first experiment is not strong evidence that the children were actually making elemental transitive inferences, as in this experiment the children were allowed to see the actual sizes of all the rods. But their second ("no visual feedback") experiment was designed to obviate this problem by never showing the children the actual lengths of the rods. Even so, we feel that this second experiment cannot offer convincing evidence for the existence of their elemental transitivity notion in 4 yr old children because we are able to propose two other types of strategies which could have been adopted and which would succeed equally well without requiring the idea of transitivity. These strategies and the experiments we performed to test whether they are being used (experiments II, III and IV) are described here, but first we describe experiment I, in which we replicate Bryant and Trabasso's second experiment and investigate their claim that memory limitations play an important role in transitivity experiments.

In experiment I, only a slight difference in memory criterion distinguishes our experiment from Bryant and Trabasso's second ("no visual feedback") experiment. The material consisted of five sticks of decreasing lengths, each one of separate colour. The sticks were presented protruding slightly above a screen which hid their actual lengths. Beginning with the first adjacent pair AB, and going on through BC, CD to the last pair DE, or alternatively starting with the last and going to the first, the children were trained to point out the bigger and smaller stick of the pair. Ten children were trained in increasing order, and ten in decreasing order. Each pair was trained individually until a criterion of eight out of ten correct choices had been obtained, and then the experimenter passed on to the next pair.

The second phase of the training period began after all the pairs had been learnt to criterion; the series of four pairs were shown in a random sequence till on each pair the child had responded correctly twice in succession. (This is where the difference with Bryant and Trabasso occurs: their performance criterion was six times in succession.) At no time were the children shown the actual lengths of the sticks.

In the test phase of the experiment, the children were shown all possible pairs of sticks, including those non-adjacent ("transitive") pairs on which they had not been

trained. The ten possible pairs were shown in random order four times each, giving a total of forty responses per subject. The child was not informed if his choice was correct.

The experiment was performed on a group of children of about the same age (4 yr 1 month) as Bryant and Trabasso's youngest age group (4 yr 5 month).

Table 1 Percentages of Correct Choices ($A > B > C > D > E$) for Twenty Children, aged 3 yr 10 month to 4 yr 4 month (Mean Age 4 yr 1 month) in Experiment I

	B	C	D	E
A	73	86 (61) *	93	97
B		84	82 (64) *	97
C			76	92 (70) *
D				92

Four responses on each pair were made by each child.

All scores are significantly above chance, as assuming that the four individual responses of a child on each pair are independent, the score for twenty subjects on any pair differs from chance at the 0.001 level if it exceeds 67.3% (χ^2 test).

* Limit values predicted by Bryant and Trabasso's calculation according to which correct choice on a transitive pair might require independent recall of both underlying pairs: $P_{BD} \leq P_{BC} \times P_{CD}$. Using this hypothesis the actual scores found should be less than the values in parentheses. This is clearly not the case.

The results, shown in Table 1, are compatible with those of Bryant and Trabasso, all scores being better than chance at the 0.001 level. But two aspects of our data cast doubt on Bryant and Trabasso's interpretations.

First, the performance on the unlearned pairs shows that the children perform much better than one would predict if they had to remember and combine the individual adjacent pairs to make a decision.

Table 2 Comparison of Experiment I (Ours) with Bryant and Trabasso's (B + T) Results for Twenty Children (Mean Age 4 yr 5 month)

	Correct choices for unlearned pairs (%)					
	AC	AD	AE	BD	BE	CE
Ours	86	93	97	82	97	92
B + T	98	93	97	82	90	88
B + T - Ours	+12	0	0	0	-7	-4
	Correct choices for learnt pairs (%)					
	AB	BC	CD	DE		
Ours	73	84	76	92		
B + T	98	89	87	94		
B + T - Ours	+25	+5	+11	+2		

Four responses per pair per child.

Second, in comparison to Bryant and Trabasso's results, our decreased learning criterion seems to have worsened the performance only on the learnt pairs (Table 2). This fact does not fit very well with the Bryant and Trabasso idea that it is because memory deficits were avoided in their experiment that the young children succeeded, for under this hypothesis, reducing the learning criterion should particularly have affected the non-adjacent, transitive pairs in our experiment.

Both these aspects of our data indicate that the method used by the children does not depend very much on remembering the learnt pairs, a fact which is difficult to understand if the children are making transitive inferences. As in addition the children executed the task with surprising ease, we felt that we might do well to search for the existence of possibly simpler alternative strategies before claiming that the observed behaviour was the result of a transitive inference independent of the memory of adjacent pairs.

Our subsequent experiments were designed to test whether alternative strategies related to the order of learning of adjacent pairs or the grouping of the sticks by "labels"

might explain the results. We used children between 3 yr 10 month and 4 yr 6 month (mean 4 yr 3 month) being slightly younger than the 4 yr 5 month of Bryant and Trabasso's youngest age group. We retained the criterion of learning used in our experiment I.

It is possible that, as Bryant and Trabasso presented sticks in ascending or descending order, the children in their experiment remembered the order of occurrence of new sticks seen in the sequence of presentation. Thus every new stick seen would be added to a list such as "blue, black, red, etc. . .". In the test phase of the experiment, the child only needs to check which of the two sticks he is shown occurs first in the list. In the case of decreasing presentation, this will be the biggest stick, and in the case of increasing presentation, it will be the smallest.

In experiment II we modified the technique used before so that during all phases of the training the pairs of sticks were presented in random rather than ascending or descending order, for example EC DE AB CE, or AB CD DE BC.

The results of the test are shown in Table 3. There is clearly no evidence that the children do worse in this "disorder condition". This failure to diminish performance in the test goes against the idea that the children are remembering the order of presentation of the sticks.

Table 3 Presentation in Random Order of Forty Children (Experiment II) aged 3 yr 10 month to 4 yr 6 month (Mean Age 4 yr 3 month)

Correct choices A > B > C > D > E (%)				
	B	C	D	E
A	73	83	87	92
B		88	81	90
C			84	96
D				94

All scores significantly different from chance at 0.001 level (see Table 1).

Four responses per pair per child.

On the other hand, the data do not seem to undermine the claim that the children are able to make transitive inferences in the conditions of the Bryant and Trabasso experiment. In a control experiment, however, we found that, in contrast to children, adults do considerably worse in the disorder condition than in the order condition (Table 4). We can conclude that children implement transitivity differently, and sometimes better than adults. Alternatively, and more consistent with the data presented below, we can suppose that the children in these experiments were not using transitivity at all.

The hypothesis of the 'labelling strategy' seems to be complicated at first sight, but is in fact simple, being derived from the idea that in young children the notion of size is absolute rather than relative. It is proposed that the child does the following three things during the training phase of the experiment. First he remembers each pair as a "situation" in which he can make the correct judgment: when confronted with rods A and B together, he says A is bigger and B smaller. When confronted with B and C together he says that B is bigger and C smaller, and so on. He makes no effort to combine these individual situations, as to him they do not conflict.

Second, he attaches absolute labels to the rods: if in a given situation a rod is longer, it gets the label "big" if it is smaller, it gets the label "small". Rods which can be both big and small retain no useful label. Thus, at the end of the training phase, the rod A has the label "big", and the rod E has the label "small", while the rest are "nonentities".

The third hypothesis is that if a rod has occurred in a situation with a rod that has a given label, it may acquire the same label. Thus the rods B and D are not always "nonentities", as B can become "big" and D can become "small" by virtue of having been presented with A or E.

What the child retains from the training phase of the experiment is therefore a memory of the correct answer to each pair of adjacent sticks; a "labelling" in which stick A is considered "big", stick E is "small"; and from the third hypothesis one or both of the following: that stick B is also "big" (a) and/or that stick D is also "small" (b).

Summarising the result of this procedure the sticks fall into three groups: the "big", the "small", and the "nonentities". Depending on what version of the third hypothesis is used the sticks will be differently distributed in the groups. When stick B is also "big" (a) the grouping will be "big" AB, "nonentities" CD, "small" E; when stick D is also "small" (b) it will be "big" A, "nonentities" BC, and "small" DE; and with both (a+b) it will be "big" AB, "nonentities" C, and "small" DE respectively.

Given any two sticks, the child can use one of two methods to make the correct response. If they are adjacent, he needs only remember the response he was taught to make on that pair. If they are not adjacent, at least one of them will belong to the big group, or to the small group, and so will be considered bigger, or smaller, than the other stick.

Clearly this "labelling" strategy cannot be considered as transitivity. Yet it is perfectly consistent with all the data assembled above. Indeed certain aspects of the experiments discussed above are better dealt with than if we assume that the children are making transitive inferences.

In particular, because of the reduced training given in experiment I, during the test the children may have had difficulty remembering the comparisons they were taught, and may thus have resorted to using labels instead. As, by virtue of the third hypothesis, two adjacent sticks may in fact have the same labels, confusions must have arisen on these pairs. This explains the fact that whereas reducing the training criterion did not affect the performance on unlearned, transitive pairs, that on learnt pairs was diminished compared with the Bryant and Trabasso results.

The idea that confusions can arise on pairs having the same label enables us to determine whether hypothesis a, b or a+b is more common. Looking over the success rate of all pairs over all types of presentation we find that grouping seems to occur mainly according to hypothesis a, that is AB, CD and E. Thus AE, BE, CE and DE (comparisons with the smallest stick) fall into a group having the highest scores. AC, AD, BD and BC (comparisons with "nonentities") have lower scores. The comparison AB between the two "big" sticks has the lowest score of all. It will be noted that the same grouping, with the smallest stick standing alone, recurs in all our experiments.

An essential aspect of the "labelling" strategy described above is that we must make the assumption that during the test the child is sometimes content to take his decisions on the basis of the label of only one of the sticks, the other one being a "nonentity", which is neither "big" nor "small". We made use of this fact in our third experiment, which was designed to determine if there was actually positive evidence that the children were using the "labelling" strategy or some derivative of it.

Table 4 Comparison of Adults' and Children's Performance in Ordered and Disordered Presentations

Total number of correct answers in the test (maximum score=40)				
	Ten subjects in disordered presentation		Ten subjects in ordered presentation	
	Median	IQR	Median	IQR
Random adults	25	5	40	0
Children in experiments I and II	34.5	2	35.5	2
				Significance of difference between the two types of presentation *
				0.05
				n.s.

* By χ^2 test of the generalized median.
IQR, Interquartile range.

Table 5 Ten Children (Experiment III) aged 3 yr 10 month to 4 yr 4 month (Mean Age 4 yr 1 month)

	B	C	D	E
A	72	88	92	89
B		60	70	80
C			46	80
D				89

Four responses per pair per child. % "correct" choice relative to the hypothetical ordering $A > B > C > D > E$.

for him to assume is either $A > B > C > D$ or else that $C > D > A > B$. Under this hypothesis then, and in contrast to the "labelling" hypothesis, if the child chooses $A > D$, he should make $B > C$.

The results of the experiment are shown in Table 6. It is seen that the tendency is to consider $A > D$ and $B < C$, thus confirming the prediction made according to the labelling hypothesis.

Because the young child is unable to say directly what he knows, the researcher studying abstract ideas is forced to evaluate the child's ability indirectly by means of the child's

Table 6 Percentage Scores given Relative to the Hypothetical Ordering $A > B > C > D$ of Thirty Children aged 3 yr 10 month to 4 yr 6 month (Mean Age 4 yr 2 month) (Experiment IV)

Pair	Score	Predicted score according to labelling hypothesis	Number of children making 7/8 or 8/8 choices so that both A>D and B<C	Mixed case †	Predicted score according to ordering hypothesis			
					Case A>B>C>D	Number of children making 7/8 or 8/8 choices so that both A>D and B>C	Case C>D>A>B	Number of children making 7/8 or 8/8 choices such that both C>B and D>A
A>B	88 *	100	15	100	100	None	100	None
A>C	52	50		50	100		0	
A>D	85 *	100		50	100		0	
B>C	27 *	0		50	100		0	
B>D	50	50		50	100		0	
C>D	87 *	100		100	100		100	

* Significantly different from chance at 0.001 (see Table 1).

† Combining random choice of $A > B > C > D$ and $C > D > A > B$ for all thirty children.

In this third experiment, we proceeded as before, except we simply left stick C (always a "nonentity") out of the training phase, and trained the children on pairs AB, BD and DE. But in the test stage, the middle stick was used exactly as in the preceding experiments.

We predicted that stick C, never having been seen before, would fall into the group of "nonentities". Our prediction was confirmed. The children showed no surprise when suddenly confronted with stick C in the test, and they gave the responses shown in Table 5.

The labelling idea is clearly compatible with the results. Stick C is generally inserted between sticks A and E. Its position is slightly more often confused with D than with B, suggesting that after the training with four sticks, the grouping AB DE was preponderant.

It is important to note that, as they contain pairs which are logically undecidable, this and the following experiment cannot give direct evidence against the hypothesis that the children possess the idea of transitivity. But inasmuch as the results of these experiments go against a hypothesis which might be considered to be derived from or a prerequisite for the idea of transitivity, that is to say the ordering hypothesis, and inasmuch as they do support the labelling hypothesis in general, it becomes plausible that the labelling hypothesis might also be used in the transitivity experiments I and II.

As a further test of the "labelling" hypothesis, we performed an experiment with only four sticks (experiment IV), in which the training was considerably simplified. Only two pairs of sticks were presented, namely AB and CD. Using this condition the children know that A is bigger than B, and that C is bigger than D, but they have no way of knowing the relation between A and C, or between B and C.

On the "labelling" hypothesis, as both A and C have the same label ("big"), and as both B and D are "small", we would always expect the child to consider $A > D$ and $B < C$.

Alternatively, one might expect that a young child possessing the notion of transitivity or using any kind of "ordering method" would impose an order on a set of sticks even when there is insufficient information to know the correct order. Thus, in this experiment, the most obvious thing

behaviour. This means that it is difficult for any single experiment to bring unequivocal evidence for the existence or absence of a given cognitive capacity. Just as failure can always be attributed to difficulties such as with material or memory overload, success can always be brought about by the use of some kind of material-orientated heuristic which happens to work in the given situation.

In their experiment to show up transitivity in young children, Bryant and Trabasso used an ingenious method to obviate mnemonic and manipulation difficulties. But they omitted to check that success in their experiment could not be brought about by other strategies.

In our experiments we have found that Bryant and Trabasso's idea that their children succeeded because a memory difficulty had been overcome is not entirely convincing. Our results also indicate that a "labelling" strategy in which no idea of relative length or order is needed cannot be excluded as a possible heuristic in the Bryant and Trabasso task. Indeed, predictions made according to this hypothesis are more consistent with the data than the idea that the children are making transitive inferences.

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BOOK REVIEWS

Pigmentation and Evolution

The Evolution of Melanism: The Study of a Recurring Necessity, with Special Reference to Industrial Melanism in the Lepidoptera. By Bernard Kettlewell. Pp. xxiv+423. (Clarendon: Oxford; Oxford University: London, August 1973.) £10.25.

H. B. D. KETTLEWELL is the world authority on the experimental study of melanism in the Lepidoptera. It was he who pioneered this subject, and much of the subsequent field work by others has been based on his original experimental techniques. The publication of this book is therefore quite an event.

The book contains not only an account of many aspects of melanism but also detailed descriptions of the author's massive contribution to our knowledge of the phenomenon in the Lepidoptera. He recounts in detail his ingenious experiments which first demonstrated intensive selective predation by birds on moths, which largely accounts for the presence of melanics in both industrial and some non-polluted rural areas. Thus, his comparative study shows that melanics can be at an advantage in certain ecological circumstances unassociated with industrialisation, and that identical phenotypes can be advantageous in both industrial and non-industrial conditions. He details his studies into the rare phenomenon of a polymorphism where the melanic is a recessive and not a dominant. The author shows that melanism in the Lepidoptera is a very old phenomenon. This conclusion, coupled with his genetic work on the control of dominance in the peppered moth, leads him to postulate that the present dominance relationships between melanic and non-melanic forms are due to the evolution of dominance in the distant past.

The text of this book makes extremely interesting reading and will fascinate the general reader concerned with the study of microevolution, for there is a great deal of information packed into it. The specialist will, however, find the book quite difficult in places for several reasons. First, there is a number of typographical errors (the largest I have ever seen in a scientific work), some of which are confusing. Second, many of the plates and figures have quite inadequate legends, nor are they properly explained in the main body of the book. Third, the most ap-

propriate references are frequently not given, and the text does not agree with the appendices. These criticisms can be illustrated by a selection of examples. Thus the symbols < and > are confused in many places. On page 181 *Xylophasia exilis* is said to be found in many areas over 100 feet on the north Scottish mainland. I wonder if this is a misprint for 1,000 feet. On pages 316–317 it is stated that 94% of the gene-complex of a hybrid brood, described as an F₄ between British and Canadian stock, must be of Canadian origin. This is clearly wrong, surely it is the fourth backcross. On plate 15.1, No. 6, the butterfly is said to be a female but is clearly a male. Perhaps one of the most inadequate legends to a figure is to be found on page 204. The figure is entirely useless since we are not told how it was constructed, for example with respect to the daily survival rate used.

At the top of page 136 Kettlewell refers to the cline in the frequency of melanics of the peppered moth from Liverpool into North Wales and cites a paper by Clarke and myself (1963), whereas the most up to date reference to this study is 1966. The author certainly knows of the latter paper since it is referred to at the bottom of the same page but in a different connection. However, he does not refer to these extensive data in appendix C when he is recording the frequency of melanics in various localities in Britain. There is a number of serious discrepancies between data quoted in the text and those given in appendices B and C. Consequently one does not know which values to believe. Furthermore, when discussing exceptionally high frequencies of melanics in *Gonodontis bidentata* it is not clear why the author picked Leeds, since a paper by Askew *et al.* (1971), which appears in the bibliography, shows that it reaches an even higher frequency in parts of Manchester. Unfortunately in appendix B it is not made clear that the actual location within a town is important when considering this species, and Manchester is listed as having a frequency of melanics of 55.4%, and no reference is made to the more accurate data of Askew *et al.*

The author gives an extensive bibliography. It would, however, help the reader if he indicated which entries were cited in the text and which were given as additional reading.

Perhaps the most serious criticisms

of the book stem from two remarks in the preface. First, the author says: "Designs of experiments laid down in advance from armchairs by biometricians, without intimate knowledge of the intricacies in the field, frequently failed." However, some statistical advice might on occasion have been useful. Thus when one examines the experiment mentioned on page 283 in which a monomorphic colony of *Panaxia dominula f. bimacula* was deliberately established, one finds that two of the avowed four objectives of the experiment—(1) to compare the behaviour of the *bimacula* and the *typica* forms, and (2) to study possible differences in the reaction of the predators to these two forms—cannot be achieved by the design of the experiment. This is obvious since the genetic constitution of the colony will be unique. Consequently any conclusions about the comparative behaviour of the two forms based on the *monomorphic colony* will be invalid, since both forms with a similar genetic background are not available to study. Furthermore, the reaction of the predators could only be observed with respect to one of the forms, the other being absent.

The second difficulty arises from the author's philosophy when he says: "Nor can I see any advantage in re-writing the techniques and results of experiments which I consider were adequately presented at an earlier date". This attitude has led him into serious difficulties. The figure on page 145 purports to be the relationship between the frequencies of *insularia* plotted against *carbonaria* frequencies. It is clear that this cannot be so, since populations with 90% or more *carbonaria* sometimes have frequencies of *insularia* in excess of 40% in the diagram. In the body of the text we are told that the curve refers to the estimated gene frequencies of the two forms. But this also cannot be right since on page 142 the author states that the two forms are, on occasion, controlled by allelomorphs of the same locus. It transpires that the diagram has been taken from Kettlewell's 1958 paper, where it was assumed that the two loci were independent. Since the text has not been changed from the original paper in order to take this into account, this and the figure are useless, even if the conclusions by some happy chance may be correct. Incidentally, we are told that the diagram is

based on the data from appendix C, but in fact it ignores all the data collected since 1956. The figure showing the British distribution of melanics, page 135, has also been lifted bodily from the same paper and has not been updated, thus it loses a great deal of its value.

Despite these serious criticisms, and many more, the volume is clearly essential reading for students of micro-evolution. One of its very great merits is the large number of speculative conclusions which have never been published before, and which will be a fruitful source of future experiments for many years to come. If the author had taken a great deal more trouble with the book and the publishers had made sure the plates were inserted in the right places (they have not in my copy), this book, instead of being a very interesting and useful volume, would have been a brilliant one.

P. M. SHEPPARD

Estimating Animals

The Estimation of Animal Abundance and Related Parameters. By G. A. F. Seber. Pp. xii+56. (Griffin: London, May 1973.) £12.

OFTEN a first problem in ecology is to devise a census technique. Rarely are direct absolute counts possible and recourse must be made to sampling methods. Many of these have been derived by statisticians and mathematicians and they answer the purpose just so long as the simplifying assumptions on which they are based are applicable. This book, written by an authority in statistics (indeed the author is Professor of Statistics at Auckland University), brings together the various mathematical techniques for estimating animal numbers and such related parameters as birth, mortality and migration rates. It is certainly useful to have all these techniques presented as a reference text which adopts a standardised notation, defined in a preliminary chapter, but with this somewhat esoteric content the book is rather expensive. Its value to a practising ecologist must depend on its accuracy, and its completeness, and perhaps also on any critical discussion of the validity and scope of the techniques mentioned.

On the first point the book certainly scores, for the field has been surveyed comprehensively and well. Each major topic, for example, the chapter dealing with "mark releases before the sampling period in open populations", is broken down to such categories as "single release: instantaneous sampling" or "single release and continuous sampling: mortality estimates" and under this last head maximum-likelihood and regression estimates are considered. In this way, and throughout the book, the final

classification usually resolves into a particular mathematical technique defined by equations and followed by one or two worked examples from the literature.

Printers and bad proof reading can make 'hash and blotchy' out of mathematical equations and symbols—indeed, some of the original published methods have appeared with irritating errors such as wrong signs. At a quick check I have found no errors in the formulae but my search has not been exhaustive.

On the third point, the book is a bit disappointing, for it offers no real discussion of the validity of the underlying biological assumptions. In capture-recapture studies it is invariably a necessary assumption that every animal in the population, whether marked or unmarked, has the same probability of being caught in the i -th sample, assuming it to be alive. Real animals rarely satisfy such premises. In fact, it might be suggested that the essence of survival has been the evolution of predictive mechanisms, of information exchange through communal behaviour, of social hierarchies which restrict movement, indeed of a host of conventions which serve to remove any element of chance and therefore confound our simple premises that individuals can be assumed to behave randomly. Perhaps we need some new approaches. In the meanwhile, this book will serve as a helpful reference work for numerate experts.

R. K. MURTON

Statistics in Medicine

Causal Thinking in the Health Sciences: Concepts and Strategies of Epidemiology. By Mervyn Susser. Pp. xviii+181. (Oxford University: London and New York, August 1973.) £2.20.

ONLY the first part of the title of this book appears on the cover, on the fly-leaf, and on each page. This gives a most misleading impression. It leads one to expect a proposal of some novel mode of reasoning, or perhaps a polemic on the inadequacy of current modes of reasoning, about medical practice. Indeed, I missed the title page and read with initial prejudice and with growing surprise. For in fact the book is a systematic examination of epidemiological methods in medicine, that is, of using statistical data to establish the existence of hazards to health and the means of preventing them. This is the discipline that has to its credit the discovery of the means of eliminating cholera before the cholera vibrio was known; the discovery that most bronchial cancer, and most chronic respiratory disease, is linked in some as yet unknown way to the smoking of cigarettes and can be avoided by avoiding such smoking; the discovery

that asbestos is capable of causing, by as yet unknown means, cancers of the mesothelial layers of the lung and other viscera.

Epidemiology has also discovered, however, associations between the softness of domestic water supplies and mortality from ischaemic heart disease, and between potato blight and births of children with spina bifida. Whether these associations reflect causal links is still a matter for dispute, and conflicting evidence has been produced. Professor Susser's concern is with the nature of epidemiological evidence, its classification into types, and the discovery of means by which direct causal links can be distinguished from indirect ones and from associations with no causal element at all.

The first six chapters provide an introduction to epidemiology and to the complex interplay between the environment and the population inhabiting it. The discussion is general, though cogent examples are given, often in the form of what the author calls "exhibits"—tabular or graphical data displayed as if on a wall-board. The development is clear, but heavy going and handicapped by technical terms of low mnemonic value which are introduced as new factors are recognised to enter the epidemiological situation. One finds, and can in part translate with the aid of the index, sentences such as "The characteristic that distinguishes the component variable from other control variables is its integration, by definition or implication, with the global independent variable under study". Mercifully they are uncommon.

In the remaining five chapters the strategies available to the epidemiologist to elucidate complex associations are examined systematically. These chapters are stimulating and thought provoking, and they succeed to a great extent in reducing to order the variety of difficulties that beset this process. No general principles emerge for recognising the disturbing factors that may be present in a particular case, and the student must learn by analogy to try to guess what they may be by noting what in other cases they have been. Once again, however, the naming of these factors is unhelpful: "When a moderator variable is introduced into an analysis, and reveals interaction, the process is called specification". Incidentally, interaction is here defined as the difference between the mean effects of one factor when another is present or absent. This is twice the size of the interaction according to the conventional statistical definition.

The book can be strongly recommended, not only to epidemiologists, but also to those interested in the findings of epidemiology. It represents a major advance in the process of establishing

standards of rigour for epidemiological arguments, and should generate much fruitful debate. I would, for instance, argue the wrongness of the statement "In the social and biological world, the more general and mathematical a formulation the less it is likely to represent the parochial realities of our concern". Are we really more interested in predicting a local epidemic with precision than with giving insights into the process of its spread? P. D. OLDHAM

Skye is Only the Start

Past and Present Vegetation of the Isle of Skye: a Palaeoecological Study. By H. J. B. Birks. Pp. xi+415. (Cambridge University: London, June 1973.) £13.50; \$39.50.

THIS book is without precedent in the history of plant ecological literature both in its intensive examination of the flora of a relatively small area and in its attempt to marry palaeoecological studies with a survey of modern flora. Although its title might suggest a highly specialised work of limited appeal, the book contains several sections which are of considerable relevance to many ecological fields.

There are two main sections to the work, the first being a detailed survey of the present vegetation of the Isle of Skye and the second dealing with the author's palaeoecological investigations of certain sediments on the island. The first section occupies more than half of the book, mainly as a result of ninety-six pages of phytosociological tables included within it. These tables document an extensive survey of the island's vegetation carried out by recording 551 four-metre-square quadrats, selected subjectively to ensure complete coverage of the island's diverse habitat types. The results are arranged in tables resembling those of the Braun Blanquet continental system of phytosociology, differing only in the absence of sociability indices. The classification and nomenclature of "vegetational units" also follow the continental system.

Many British ecologists will object to the use of subjective techniques and also the application of the continental 'pigeonhole' philosophy in classifying stands. One must, however, acknowledge the efficiency of these methods for primary vegetational survey. Continental phytosociologists may feel that some of their carefully defined association binomials have been applied a little loosely to some of the very distinctive, oceanic plant communities of Skye. The author has declined to erect new associations on the grounds that too little is known of the fidelity of species within the vegetational units he has recognised. Without such a know-

ledge, however, he may have been unwise in assigning some of them to existing associations mainly described from the continent.

The palaeoecological section of the book contains a very valuable chapter on pollen identification. There are also extremely useful data on surface pollen spectra from different vegetational types which aid the interpretation of the fossil pollen assemblages. Pollen analyses are given for sediments from five sites on Skye which were chosen to provide representative examples of vegetational history in a region of diverse topography and geology. The diagrams are zoned on a local assemblage zone basis, but are interpreted as representing the classical late Devensian sequence, including the interstadial phase (Allerød). Undoubtedly some people will question this interpretation. *Betula* pollen is present in very low quantities in the basal sediments and does not provide clear evidence upon which zonation can be based, except possibly at Loch Meodal. *Juniperus*, on the other hand, does exhibit the expected double peak associated with the complete late Devensian sequence. My feeling is that Birks is probably correct in his interpretation; however, it is regrettable that he does not possess more radiocarbon dates from lower (interstadial) levels. At present the temporal correlation chart (Fig. 27, page 352), which summarises Birks's conclusions, must be regarded as speculative.

This is a remarkable book describing the results of a great deal of painstaking and accurate work. It is a volume which will undoubtedly have a profound influence upon the future development of Quaternary plant ecology in the British Isles. PETER D. MOORE

Urchins and Cucumbers

Experimental Embryology of Echinoderms. By Sven Hörstadius. Pp. 192. (Oxford University: London; Clarendon: Oxford, September 1973.) £5.

FOR more than forty years experimental embryologists have recognised Sven Hörstadius as a master. From time to time he has displayed his outstanding skills on other material, but the analysis of sea urchin development has been his major and continuing interest. He now offers a historical review, informed by a lifetime's familiarity, and ruthlessly down to earth in its approach. Starfish, sea lilies and sea cucumbers have been less conspicuous in experimental work, but are appropriately mentioned.

Hörstadius deliberately avoids any detailed consideration of a number of important fields—fertilisation and the molecular biology of eggs and embryos, for example—in order to concentrate upon his main theme. This is experi-

mental morphology as investigated by the classical manipulative techniques of isolation and transplantation, and 'simple' chemical treatments. The story is well known in outline, and is very impressive. It is useful to have it brought up to date in so authoritative a way.

The example of Driesch may have discouraged later echinoderm embryologists from grandiose speculation, but it is a pity that Hörstadius is so very self controlled. It would have been of interest to know more of his views, however tentative, on the general issues that this work raises. Even the double gradient which has been so helpful in interpreting experimental results must arise, must be maintained, and must influence the expression of genetic information. To understand how would be of universal value for developmental biology. Hörstadius's questions are usually less general. It is wholly characteristic that his book should end with one that smacks more of the laboratory than the seminar. An experiment is briefly described. The expected result is not obtained. "Instead, the upper half moved down along the lower partner until the endodermal regions fused. What kind of reaction and what forces caused this strange migration?"

D. R. NEWTH

More Quanta

Theoretical Physics: an Advanced Text. Volume 3: Quantum Mechanics. By Benjamin G. Levich, V. A. Myamlin, and Yu A. Vdovin. Translated. Pp. xx+621. (North-Holland: Amsterdam and London, 1973.) Dfl. 120; \$37.50.

THE writing of a book on Quantum Mechanics, at the present time, requires a certain temerity on the part of the author. Only a messianic belief in the originality and individuality of one's approach could justify the adding of yet another volume to the already overcrowded shelves.

The present volume is neither original nor particularly individual, and its sole *raison d'être* seems to lie in the unwarranted conviction that every course on Theoretical Physics should possess its own volume on Quantum Mechanics.

One of the basic problems is to try to identify the audience it might serve. It is much too advanced to be used as an introductory text for undergraduates, even for those specialising in theoretical physics, and it does not match, say, Landau and Lifshitz for excitement and power, as an advanced treatment.

The subject matter is rather standard, with the exception of the last chapter entitled "Fundamentals of the Theory of Elementary Particles", where an interesting attempt is made to introduce the reader to quite advanced ideas which are right in the forefront of present day

research. It makes fascinating reading. In the foreword, we are also promised detailed applications to solid-state theory, but these have failed to materialise.

Some topics are rather nicely handled. The standard treatment of potential wells and barriers is enhanced by the study of some of the numerical magnitudes of the effects. The use of integral equations and Green's functions is very clearly discussed, though the presentation is somewhat lacking in motivation. The treatment of the time-energy uncertainty relations, as is traditional in the Russian school, is good. The Periodic Table and Molecular Spectra receive enough discussion to give one a real flavour for what is involved. The treatment of Coulomb Scattering begins with the case of the Screened Coulomb Potential. This leads to a natural introduction to the concept of the atomic form factor, and also avoids the mathematical and conceptual complexities associated with the pure Coulomb force. The seemingly mysterious agreement between the quantum and classical calculations of the Rutherford formula, usually not commented upon, is explained. The derivation of the classical limit of scattering theory is a noteworthy inclusion. The treatment of symmetry properties, in particular of the group $SU(2)$, is unusually good, in that it uses basic Lie Group methods, applicable to more general cases, and does not capitalise on the special and rather atypical properties of $SU(2)$ itself.

On the negative side it has to be said that the description of "wave-particle duality" is clumsy and that the whole introductory chapter, which is wide ranging and far from simple, would be incomprehensible to a newcomer to the subject. Matrix algebra is discussed in detail, yet for an understanding of the δ function one must turn to Volume 1 of the course—a strange choice of priorities. The Hamilton-Jacobi equation is introduced, yet inadequately explained. There is a long discussion of α decay and of the sensitivity of barrier effects to the energy, yet no numerical estimate to illustrate it. The derivation of degenerate perturbation theory, always a tricky question, is quite unclear, and there is in general far too little illustration of the use of perturbation theory. A fair-sized portion of the book is devoted to Relativistic Quantum Mechanics (Dirac equation, Weak and Strong interactions, Feynman diagrams). Here one has the feeling that the reader will pick up only a very superficial idea of what is involved.

The reader will be confused, after having being led through the seemingly general proof in Section 73 that the dipole moment of an atom is zero, to learn somewhat later, and without explanation, that "As distinct from

atoms, molecules may have a mean dipole moment".

The translation sometimes leaves much to be desired. "Causality" and "determinism" are confused, as are "picture" and "representation"; "measurement" is used instead of "dimension" and "rank" of a matrix for its "dimension". Some phrases are particularly outlandish: "We digress from other quantities . . ."; "The trace of a scalar is equal to its quadrupole value"; "The reaction $p + \gamma \rightarrow n + \pi^+$ representing the elementary act of the nuclear photoeffect was observed"; and so on.

Also the notation is occasionally non-standard, and it is inexcusable that no effort was made, in translating the volume, to comply with Western usage. Examples are: $\{ \}$ for the commutator bracket, $\hat{R}_x$ for the operator generating displacements along the X axis, and $*$ used sometimes as hermitian conjugate and sometimes as complex conjugate.

By and large this is not an attractive book. It is neither fish nor fowl, and aside from the rather nice treatment of a few selected topics, it is hard to see to whom it may be recommended as a whole.

E. LEADER

Art of Bonding

The Shape and Structure of Molecules. By C. A. Coulson. Pp. 85. (Clarendon: Oxford; Oxford University: London, June 1973.) £2.75 boards; £1.50 paper.

THE aim of this book is "to show how a few quite simple ideas, not particularly abstruse or mathematical, have been able to rationalise a large part of structural chemistry". The fundamental concept of localised bonds, with their characteristic energies and electron distributions, is established for diatomic molecules, using both the Heitler-London and the molecular orbital approach. The concept of hybridisation leads to a discussion of polyatomic molecules, which is followed by a short survey of valence rules for elements in the main groups of the periodic table. The final chapter gives a very brief discussion of delocalised bonds.

A book of this length cannot do more than introduce so vast and subtle a subject. The understanding of bonding is an art at least as much as a science, and only experience can teach us which approximate or qualitative treatments are useful and what is the likely range of their validity—as tested, for example, by predicting both the correct shape and stability of molecules and the correct electronic distribution obtained from X-ray scattering. Until one has digested the results of applying various bonding theories to known properties of a wide range of molecules and crystals, one cannot apply them with any confidence

to the unknown. For this purpose a much larger book is needed, which would discuss critically not only many more applications of the theory given here, but also other treatments of bonding, such as that of Sidgwick, Powell, Nyholm and Gillespie. Recognising this, the present book ends with useful suggestions for further reading.

To sum up, this book can be recommended as an introduction to the art of bonding theory, outlining the particular approach of a master practitioner both of that art and of the art of exposition.

T. H. K. BARRON

Isotopes in the World

Stable Isotope Geochemistry. By Jochen Hoefs. (Minerals, Rocks, and Inorganic Materials: Monograph Series of Theoretical and Experimental Studies, vol. 9.) Pp. ix+140. (Springer-Verlag: Berlin, New York, 1973.) 39 DM; \$14.50.

THIS small volume, which is essentially an up to date review of the most significant contributions to stable isotope geochemistry over the last ten years or so, is divided into three main sections. The first is an introduction which briefly covers some background material relating to isotopes in general, including isotope effects and fractionation processes, mass spectrometry, sample handling, and standards. This is followed by a more detailed discussion of isolation and preparation techniques, fractionation mechanisms, and the standards appropriate to the major elements studied in stable isotope geochemistry: H, C, O and S. Other less studied elements such as Se, B, N, Si, Cl, Br, Li, K, Mg and Ca are briefly mentioned. Finally, in the third and major section, a discussion is given of the variations in stable isotope ratios in various types of geological materials, extraterrestrial samples, the water cycle, and in the atmosphere and biosphere.

Overall the text is easy to follow, largely as a result of the crisp style and of the economy of words, which allow the significant contributions in a comprehensive bibliography of about 500 references to be covered in a relatively short space.

There are only a few typographical errors, for example in the equation on page 35. My only other criticism is that the author has omitted to mention one very active area in stable isotope geochemistry: the lunar rare gas isotope studies which have contributed much to the understanding of the dynamic processes which have occurred on the Moon's surface.

This book should provide invaluable reference work for many who teach and do research in geochemistry.

J. R. MAXWELL

CORRESPONDENCE

Television

SIR,—It is a relief to discover that *Nature* is beginning to watch the television screen with other than a conservative or jaundiced eye. For the most part, your recent writers have understood the weaknesses, strengths, and technical limitations of the medium. Nevertheless, I suspect that some critics are piqued that the programmes are not wholly satisfying to professors of genetics or readers in physics. I feel they might be unaware of the esoteric nature of the group to which they belong. Science programmes reach a narrow band of the potential audience, but this is vast compared with that touched by any other medium which vulgarises science. Television is laical. It is perfectly possible to use it to put across esoteric ideas in an esoteric fashion; however, to do so would be an uneconomic use of the medium. There are better ways of communicating complicated concepts.

In the past, views have been expressed that science misrepresented on television programmes will distort the public's understanding of the function and aims of scientists. This might be so. But it was worrying to see the boot on the other foot in the recent BBC Controversy programme "Soviet Scientists". Elderly members of the scientific establishment shamelessly (or blindly) manipulated a programme into a piece of propaganda. On that showing, the BBC and its attempts at political impartiality and objectivity has more to fear from science than scientists have from television.

Yours faithfully,

ROBERT REID

22 Colet Gardens,
London W14

Dr Reid was until recently Head of Science and Features at BBC TV.—ED.

DNA Methylation

SIR,—In the article by Adams¹ two papers^{2,3} from my laboratory have been misquoted. The author¹, in referring to our publications, states: "These latter experiments, however, indicated that the maximum rate of methylation occurred at about the time of gastrulation; . . .". On the contrary, we wrote², in 1965, "From these experiments we cannot conclude that the amount of 5-methylcytosine of DNA at later stages of development changes, because we have

not analysed as yet such parameters as, for example, the amount of DNA synthesised during the incubation period, the pool of methionine and the rate of synthesis of DNA at the stages indicated." And in the 1968 paper³: "No conclusion about quantitative differences of methylation at the different stages can be drawn because no information was collected on the pool of methionine and on the rate of DNA synthesis during the period of incubation with labelled methionine."

Moreover, in the article by Adams¹, reference 4 is a misprint (page 28, column 2, line 8). The right references should be 2 and 7, namely, our two *J. molec. Biol.* papers.

I wish to add a word of caution for the implications derived by Adams from a stated five-fold increase of the ratio of 5-methylcytosine to thymine in the heavy DNA¹. It is essential in all work on methylation of DNA in developing sea urchin embryos to consider the 2,000-fold increase in nuclear DNA, which occurs from fertilisation to the pluteus stage with no change in the total mass, the total nitrogen and the total RNA. An additional factor to be considered in the experiments by Adams¹ is the high toxicity of 5-bromo-2'-deoxyuridine to the embryos.

Yours faithfully,

EDUARDO SCARANO

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80125 Naples*

¹ Adams, R. L. P., *Nature new Biol.*, **244**, 27 (1973).

² Scarano, E., Iaccarino, M., Grippo, P., and Winckelmans, D., *J. molec. Biol.*, **14**, 603 (1965).

³ Grippo, P., Iaccarino, M., Parisi, E., and Scarano, E., *J. molec. Biol.*, **36**, 195 (1968).

Soviet Science

SIR,—I write, not only as a scientist, but also as a member of the Communist Party since my student days in 1932. Many illusions have been shattered during the 40 or so years since then; the process of achieving communist society is much longer and more tortuous than we believed in the 1930s. The way in which this change comes about is conditioned by the specific historical circumstances in each country.

It would take a vivid imagination to conceive of more difficult circumstances than those which the Soviet Union has

had to meet. One has only to consider that there was almost no experience of democratic forms of government on which to build; that the young Soviet Union was invaded by fourteen foreign armies and that it spent years of preparation for war in a hostile world and suffered the unparalleled destruction of the last war, to understand how it is possible for present Soviet attitudes to have developed and become entrenched.

I firmly believe, however, that great efforts should be made in the Soviet Union to open up the ways in which dissent can be expressed and taken into account, and so far, progress in this field has been disappointingly slow. In so far as administrative action is taken to inhibit the expression of dissent, I believe this to be wrong and against the best interests, not only of science, but also of socialism. It may be true that a good scientist is not necessarily a good politician, but that does not diminish his right to express his opinions on political matters.

It is also true that Soviet scientists have a lot to give the world as well as to learn from it but I do not see how they can do so unless they are able freely to take part in conferences and discussions outside the Soviet Union.

Soviet society is developing rapidly and has many splendid achievements to record. But it also has some big problems requiring solution. One of these is the correct handling of dissent, and its solution is crucial to the future of socialism.

I am confident that the problem will be solved as others have been in the past, but one thing is certain, its solution will not be helped, and may well be retarded, by the organisation of a campaign of public protest outside the Soviet Union.

Expression of concern over authenticated cases, provided it is done with understanding and in a spirit of helpfulness and, if I may say so, humility, is more likely to be effective. But there are powerful anti-Soviet propaganda machines waiting for any material which can be used to stir up the cold war and, if we provide ammunition for their campaigns, our efforts will certainly be counter-productive. This is essentially what I attempted to say in the recent BBC Controversy programme "Soviet Scientists".

Yours faithfully,

J. W. JEFFERY

*Department of Crystallography,
Birkbeck College,
University of London*

Smoking and Pregnancy

SIR,—Your invitation for Dr Yerushalmy to respond to your editorial¹ to the correspondence on smoking during pregnancy arrived a few days after his death. Dr Yerushalmy would have enjoyed contributing to the discussions which centred around the two propositions that he described²: that cigarette smoking acts as an exogenous factor which interferes with the intra-uterine growth and development of the foetus and that smokers may represent a group of people whose reproductive experience would have duplicated the observed pattern whether or not they smoked.

His study on 'Infants with Low Birth Weight Born Before Their Mothers Started to Smoke'³ seems to support the hypothesis that the higher incidence of low-birth-weight infants is due to the smoker, not the smoking. Dr Yerushalmy would probably have made a strong plea, as he did in his paper, to repeat this study on larger samples in other population groups to get a definite verdict on the alternative hypothesis. This could be done by asking large numbers of pregnant women who smoke when they started to smoke, what was the birth weight and the health data of previously born children.

Yours faithfully,

BEA J. VAN DEN BERG

*Child Health and Development Studies,
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Oakland, California 94611*

¹*Nature*, **245**, 61 (1973).

²Yerushalmy, J., *Proc. 6th Berkeley Symp. Math. Statistics Probability*, **IV**, 329 (1972).

³Yerushalmy, J., *Am. J. Obstet. Gynec.*, **112**, 2 (1972).

Smoking, Pregnancy and Publicity

SIR,—Professor Burch¹ and Dr Hickey *et al.*² rely heavily on the publications of the late Professor Yerushalmy to criticise my own remarks³. As there still seems to be some confusion about the value of his statistical evidence, I would like to elaborate a little on the points I made in my earlier letter.

First, estimates of the mean difference in birthweight between babies of smokers and non-smokers do, of course, differ, but are all in the 150 to 250 g range, and the exact value for a particular population is not relevant to the argument. Yerushalmy's⁴ test for a shift of 200 g in the mean of the birthweight distributions of babies of smokers is inappropriate.

What he should have tested for was an equivalence of the distributions apart from a shift in mean value, which is estimated from the data and need not be

exactly 200 g. As Dr James⁵ has shown, however, we will still find a reversal in mortality rates in the babies weighing 2,500 g or less, if a given percentage reduction in birthweight due to smoking is assumed, rather than a constant absolute reduction. Moreover, a simple study of the two birthweight distributions, in Yerushalmy's own study for example⁶, reveals quite clearly how an increased mortality among babies of smokers which is associated with a reduction in birthweight will lead to a lower mortality among babies of smokers if only the babies weighing 2,500 g or less are studied. For this reason all of Yerushalmy's analyses of babies weighing 2,500 g or less are irrelevant to the main issue.

Professor Burch quotes Yerushalmy's claim that there is no association between birthweight and age for mothers less than twenty-five years old. There are, however, studies other than Professor Yerushalmy's and with much larger numbers^{7,8} which do show that the proportion of low birthweight babies born to women under twenty is about 20% higher than that for women aged between twenty and twenty-four. Until due allowance is made for this, Yerushalmy's conclusions should not be accepted at their face value.

As Professor Burch observes, I am quite happy to "concede" that the scientific case for a causal relationship is not yet completely conclusive and among other possibilities we need to investigate the 'constitutional' hypothesis. The point at issue, however, is how the available evidence should be assessed, and what publicity should be given to any conclusions.

An impartial observer, it seems to me, would have little doubt that this evidence justifies efforts aimed at persuading pregnant women to stop smoking. Your correspondents, I suggest, have confused this practical, ethical issue with the other problem of scientific proof.

Yours faithfully,

HARVEY GOLDSTEIN

*National Children's Bureau,
8 Wakley Street, Islington,
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¹ Burch, P. R. J., *Nature*, **246**, 177 (1973).

² Hickey, R. J., Boyce, D. E., and Clelland, R. C., *Nature*, **246**, 177 (1973).

³ Goldstein, H., *Nature*, **245**, 467 (1973).

⁴ Yerushalmy, J., *Am. J. Obstet. Gynec.*, **114**, 571 (1972).

⁵ James, W. H., *Nature*, **246**, 235 (1973).

⁶ Yerushalmy, J., *Am. J. Epidemiol.*, **93**, 443 (1971).

⁷ Butler, N. R., and Alberman, E. D., *Perinatal Problems* (Livingstone, Edinburgh, 1969).

⁸ Chase, H. C., and Byrnes, M. E., *Vital and Health Statistics Series 3*, 15 (National Centre for Health Studies, Rockville, Maryland, 1972).

This correspondence is now closed.—ED.

Literature Citations

SIR,—The transcription of literature citations in scientific articles is rendered unnecessarily tedious by the diversity of arbitrary conventions adopted by different journals. References jotted down for subsequent bibliographical use must be differently transcribed according to the journal in which they are to appear. The differences are matters of form and style as well as of completeness.

We are writing to urge that commissions of editors convene information retrieval specialists, typographers, and some common readers to draw up broadly acceptable conventions that optimise clarity and simplicity in literature citations.

Some specific suggestions are: (1) Each scientific journal should adopt an abbreviation, if necessary, of its original or transliterated title that can be used without confusion anywhere in the scientific literature. The abbreviations of common words such as 'Annual' and 'Comptes Rendues' should be uniform. Local conventions such as the germanic use of lower case for adjectives or special self-reference terms ("This Journal") are to be avoided. (2) The presentation of author's names, initials, publication year, article title and key-words, volume number and pagination should be standardised as to sequence, punctuation and type font. Simplifications should be accomplished by deletions without rearrangement. (3) The title page of articles should bear the complete article reference as it should be cited. Fascicle numbers, issue dates and so on, not normally cited, should appear separately on the page if they serve a function for particular users.

The temporary inconvenience to journal staff in changing habits would be offset, in the long run, by diminished editorial labour in the revision of improperly submitted bibliographies.

Uniformity in these matters would cause little reduction in the marvellous particularity of individual journals and would reduce the burden on authors, readers, and especially secretaries.

Yours faithfully,

MICHAEL YARMOLINSKY

MARIE-JOËLLE HOOGVELD-LEPASTEUR

*Institut de Biologie Moléculaire,
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75005 Paris*

Bomb Disposal

SIR,—Disarming and removing explosive devices is dangerous to police and army personnel, and also to the general public. I would like to propose a

method of deactivating bombs which would considerably reduce this risk.

Liquid nitrogen is relatively safe and easily handled. A bomb sprayed with it for several minutes would be cooled to an extremely low temperature. This would have two effects. First, the battery used to detonate the explosive would become ineffective. I have verified this in the following way. Three brands of dry cell battery (Ever Ready, Winfield, Ray-O-Vac) were cooled to the temperature of liquid nitrogen and tested in a small torch. None of the batteries tested caused the torch to light. When the batteries were allowed to warm up, however, complete efficiency was restored.

Second, the detonator and explosive might reasonably be expected to be unreactive at such low temperatures. A bomb deactivated in this manner could be safely transported to a disposal site in an insulated container partially filled with liquid nitrogen.

Yours faithfully,

GEORGE DAVIDSON

K.R.U.F. Institute of Renal Disease,
Royal Infirmary,
Cardiff

Foraminifera

SIR,—The recent review of Dr J. W. Murray's book¹ by Dr J. R. Haynes

(*Nature*, **245**, 442; 1973) may have confused a number of palaeoecologists by seeming to advocate absolute abundance rather than standardised abundance for the plotting of species distributions. The latter method, now widely employed, is admittedly suspect because it makes the *a priori* assumption that all the foraminifera of an assemblage are competing.

This is not as unsatisfactory, however, as the absolute abundance method which is, of course, much affected by rates of sedimentation. As a result, plots of species using this method are often difficult to distinguish. Standardisation of data reduces this 'background noise' and furthermore enables the calculation of basic ratios (such as benthonic: planktonic or Textulariina: Miliolina: Rotaliina) which are now valuable aids to the palaeoecologist. Obviously, if the approach is biological rather than geological, then the biomass of a species per unit area is the better index.

Yours faithfully,

M. D. BRASIER

Geology Department,
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¹ Murray, J. W., *Distribution and Ecology of Living Benthic Foraminiferids* (Heinemann, London, 1973).

As suggested in my review, paucity of data and lack of control of sample material may force us to express species abundances in percentages rather than absolute numbers per unit area of volume of substrate. The desperate inadequacy of this method, however, is shown by the tables in Chapter 1 of Dr Murray's book. Not only does this method impose reciprocal relationships but the percentage figures may show a rise for a particular species between stations when the absolute numbers have fallen and *vice versa*.

At present our understanding of the autecology of foraminifer species is almost nil. Progress towards an evolutionary ecology requires knowledge of the abundance of individual species both in their habitats and when dead as part of the sediment. The variations in numbers and sorting brought about by sedimentary processes which make plots "difficult to distinguish" are precisely what concern geologists and represent the challenge that has to be met before we can properly interpret the geological record. Even where it is thought valuable simply to estimate certain crude ratios these are as easily derived from absolute numbers.

JOHN R. HAYNES

Obituary

Leonard Carmichael

DR LEONARD CARMICHAEL, Secretary of the Smithsonian Institution from 1953–64, died on September 16 in Washington, DC, at the age of 74. He was the National Geographic Society's Vice-President for Research and Exploration at the time of his death.

A distinguished physiological psychologist, Dr Carmichael also achieved renown as a science administrator in the worlds of academia, museology and government. The central unifying interest in his career, which lasted 50 years, was research: his own research and the administration and funding of the research of others.

Dr Carmichael was born on November 9, 1898, in Philadelphia, where his father was a successful physician. In 1917 he entered Tufts University, where his grandfather, Charles Hall Leonard, had for many years been Dean of the Crane Theological School. Here he became interested in zoology and psychology, and later recorded that the

two men whose books had the most influence on him as an undergraduate were the biological ultramechanist Jacques Loeb and the proponent of emergent evolution C. Lloyd Morgan.

Following graduation from Tufts, *summa cum laude*, he began graduate work at Harvard in psychology, developing an interest in a basic biological approach to the science. He later wrote that the accidental finding of a German publication of William Preyer, *Spezielle Physiologie des Embryo, Untersuchungen über die Lebenserscheinungen vor der Geburt*, in the Harvard Library, was a turning point in his life, opening his eyes to a specific area of research in his chosen field of the sensory and neural control of behaviour on which he was to spend many years. "Here at last I saw a way to investigate the topic of major interest to me—the morphological growth of the receptors and nervous system in relation to changes in behaviour as responses are released at various stages during the early ontogenetic development in each mammal

before learning begins, or at least before it becomes important."

He completed his PhD studies and theses and did a year of graduate work at the University of Berlin before beginning his teaching career at Princeton in 1924. Later he taught at Brown, where he gained his first administrative experience as Director of its psychology laboratory, and at Rochester, where he was Dean of the arts and sciences faculty. At Brown, in addition to his teaching and administrative activities, Dr Carmichael began studying the prenatal development of behaviour in mammals. With Dr H. H. Jasper he developed the electroencephalograph and published in 1935 what is believed to be the first report of such work in America.

In 1938 Dr Carmichael returned to Tufts to become, at 39, one of the youngest presidents in the college's history, but during World War II he was summoned from the campus to fill various posts in Washington. As director of the National Roster of Scien-

tific and Specialised Personnel, he organised the recruiting of scientists to work on the atomic energy and radar projects as well as other research connected to work on the war effort. In this period, Dr Carmichael later said, he spent more than a year of nights on a sleeping car between Boston and Washington. He was also President of the American Psychological Association from 1939–40.

After the war, he was a member and Vice-Chairman of the National Advisory Committee for Aeronautics, the predecessor of the National Aeronautics and Space Administration. President Eisenhower named Dr Carmichael Ambassador Extraordinary when he represented the United States at an international conference at The Hague that wrote a treaty for the protection of cultural property in time of war.

In the course of his academic career, Dr Carmichael published numerous papers on reading and visual fatigue, perceptual assimilation, the development of a kitten's ability to land on its feet, and other aspects of behavioural development related to the functions of the sense organs. Dr Carmichael also collaborated with H. C. Warren on the book *Elements of Human Psychology* (Houghton Mifflin, 1930), which was used as an introductory book for years in many major universities and colleges. They also collaborated on a *Dictionary of Psychology* (Houghton Mifflin, 1934). His later writings include *Basic Psychology*, which he wrote in 1957 to set out his point of view for the educated general reader. *Carmichael's Manual of Child Psychology*, of which he wrote part, went through a third edition in 1970.

On January 1, 1953, Dr Carmichael became Secretary of the Smithsonian, the seventh scientist to direct an institution, founded in 1846 with a bequest from the English scientist James Smithson, "for the increase and diffusion of knowledge among men". Dr Carmichael, during his 11-year tenure as Secretary, was notably successful in obtaining appropriations from Congress to foster programmes of exhibit modernisation for the Institution's museums. Under his leadership the Institution received \$36 million in Federal funds for a Museum of History and Technology, the Smithsonian's first new building in 50 years.

In 1964, at the age of 65, he insisted on retiring from the Smithsonian. He was then offered the post at the National Geographic Society. There he directed \$1.2 million in annual grants for research into sciences. His projects involved him in many activities, including the work of Dr Louis S. B. Leakey at Olduvai Gorge in Tanzania. He also worked closely with Baroness Jane van Lawick-Goodall, whose pioneering

study of wild chimpanzees broke new ground in the study of animal behaviour. Primatology was long an interest of Dr Carmichael and he had served as President of the International Primatological Congress.

He also served as President of the American Philosophical Society from April 1970–73, and was elected to several scientific organisations abroad, including the Ergonomics Research Society of the Royal Society of Arts in England, and the Société Française de Psychologie. In 1972 the National Academy of Sciences bestowed its highest award, the Hartley Public Welfare Medal, on Dr Carmichael "for eminence in the application of science to the public welfare".

Walter Rudolf Hess

PROFESSOR WALTER RUDOLF HESS, Nobel Laureate for Physiology and Medicine for 1949, the man who brought to light the functional organisation of the diencephalon, died at the age of 92 on August 22, 1973, in his home at Ascona, Switzerland.

In 1917, at the early age of 36, he became Professor of Physiology at the University of Zurich, and in this capacity Director of the Institute of Physiology, a post which he held until his retirement in 1951. He very soon became one of Europe's leading physiologists, and with great drive and energy promoted this branch of science on an international scale. Thus, foreseeing the importance of research on the adaptation of the human body to the conditions of high altitude, he founded in 1931 the international *Hochalpinen Forschungsinstitut Jungfrauojoch*. In 1938 he was delegated to organise and preside over the 16th International Congress of Physiological Sciences in Zurich. His strong personality succeeded in keeping the meeting on an entirely scientific base despite the political troubles of that time.

In his scientific work, W. R. Hess combined analytical experimentation with great synthetic power, which enabled him to view the data he obtained within the larger frame of the purposeful organisation of biological phenomena. This was already manifest in his early investigations on the viscosity of blood and general haemodynamics, but even more so in his work regarding the regulation of circulation and of respiration, concepts which were laid down in two monographs in 1930–31. It was this search for the organising principles which led W. R. Hess to begin experimentation with electrical stimulation in the brain stem—the diencephalon; his aim being to explore the autonomic

centres which govern the adaptation of circulation and respiration to the different needs of the organism during rest and work. Hess himself said that he wanted to dedicate only one year to this problem, but the work grew and was extended over twenty-five years to form what is known today as his life's work. He perfected the method of circumscribed stimulation and coagulation of subcortical structures in the unrestrained animal in 1932, and after delimitating the reactive zones in the hypothalamus concerned with regulation of circulation and respiration—results obtained in the anaesthetised animal in 1932—he proceeded to systematically explore the entire diencephalon and adjacent portions of the mesencephalon in the freely-moving awake cat.

Every physiologist is familiar with the film of Hess's cats manifesting autonomic, motor and affective-behaviour effects upon electrical stimulation within the diencephalon. Not so familiar, even today, to many a physiologist are the basic conclusions derived by Hess from his experimental work.

Hess's view of two opposing ergotrophic and trophotrophic systems was based on the following observations. Stimulation in the caudal portion of the hypothalamus, besides producing an increase in blood pressure and activation of respiration, also produces a general arousal of the animal which is associated with increased locomotor activity 'Bewegungsdrang'. Stimulation in the predominantly more rostrally lying portions of the hypothalamus and of the septum, besides yielding a decrease of blood pressure and an inhibition of respiration, produces responses which are related to food intake, digestion, excretion and thermoregulation. Thus the former effect—the ergotrophic reaction—includes both the autonomic and somatomotor responses which are activated simultaneously during effort, and therefore with expenditure of energy. The latter effect, however, the trophotrophic reaction, subserves restitution of the spent energy and guarantees the balance of the internal environment. A behaviour primarily associated with restitution is sleep, and Hess conceived sleep as a distinct type of behaviour; an active process which is governed by the central nervous system. This view has been confirmed by further research, although the narrow concept of a discrete sleep centre in the thalamus can no longer be upheld today.

The various motor effects of the head and trunk produced from the thalamus and adjacent mesencephalon are related to the regulation of posture. This subcortically organised motor system provides an indispensable support for the execution of voluntary movements by counterbalancing the forces exerted by these movements. Hess called this sys-

tem the ereismic, or supporting, motor system.

The affective-behaviour responses were obtained by Hess from the so-called intermediate zone of the hypothalamus and included the affective defence reaction (rage reaction) culminating in a purposeful and directed attack of giving way to sudden flight. Hess strongly defended the point that the rage reaction produced by electrical stimulation in the intact animal is due to an affective re-tuning of the animal and is therefore distinct from the sham rage observed in the decorticated preparation. This view has been largely confirmed today by electrical stimulation of subcortical structures in man, resulting in emotionally tuned reactions. It is mainly the pioneer work of Hess on affective behaviour which stimulated further research in laboratories throughout the world—research which today provides the neurological basis for psychology.

During his emeritus years, Hess was able to devote all his time to the subject of central representation of behaviour and psychic experience, a problem which had preoccupied him all his life. It may therefore be appropriate to close this short tribute by quoting the closing words of his last book *Psychologie in biologischer Sicht*. "... so ergibt sich die Folgerung, dass der Inhalt des subjektiven Erlebens überhaupt an den Bau des Gehirnes und die Eigenschaften der strukturellen Elemente gebunden ist und dass nur solche Bewusstseinsinhalte entwickelt werden können, welche in der Organisation des Gehirnes ihre Entsprechung haben."

Announcements

Appointments

P. N. R. Usherwood, of the University of Glasgow, has been appointed to the chair of zoology at the University of Nottingham.

Professor Israel Dostrovsky has been elected President of the Weizmann Institute of Science.

International Meetings

January 1–4, **The Association for Science Education Annual Meeting** (B. Nicholl, Esq., ASE Annual Meeting Secretary, Newman College, Birmingham B32 3NT).

January 2–4, **11th Annual Solid State Physics Conference** (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

January 3–4, **69th Ordinary Meeting of the Society for General Microbiology** (Meetings Assistant, Society for General Microbiology, Harvest House, 62 London Road, Reading RG1 5AS).

January 3–4, **Molecular Beam Kinetics Group** (Professor C. W. Nutt, Department of Chemical Engineering, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX).

January 4, **British Society for the History of Science** (The Assistant Secretary, The British Society for the History of Science, 47 Belgrave Square, London SW1X 8QX).

January 5, **The Artificial Intelligence and the Simulation of Behaviour Group** (Mrs L. Daniels, Department of Computational Logic, 9 Hope Park Square, Meadow Lane, Edinburgh EH8 9NW).

January 7–8, **Conference on Technical Aspects of Air Pollution** (Mrs Sonia Withers, Centre for Extension Studies, Loughborough University of Technology).

January 7–8, **Conference on Research in Mathematics** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY).

January 7–8, **7th Public Health Engineering Conference** (John Pickford, MSc, ACGI, MICE, FIPHE, University of Technology, Loughborough, Leicestershire LE11 3TU).

January 7–8, **The Application of Lasers to the Processing and Examination of Materials** (The Meetings Officer of the Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

January 7–9, **The Biology and Chemistry of the Cruciferae** (The Executive Secretary, Linnean Society of London, Burlington House, Piccadilly, London W1V 0LQ).

January 8, **The Annual Histochemistry Meeting of the Royal Microscopical Society** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA).

January 8–10, **Biological Clocks and Changes in the Earth's Rotation: Geophysical and Astronomical Consequences** (W. F. Mavor, Administrative Assistant, School of Physics, The University, Newcastle upon Tyne NE1 7RU).

January 10, **25th Annual General Meeting of the Institute of Biology** (Institute of Biology, 41 Queen's Gate, London SW7 5HU).

January 10–11, **Numerical Methods for Constrained Optimisation** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY).

January 14, **Measurement and Interpretation of Low Volatiles and Solubilities** (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

January 14–18, **5th International Symposium on Magnetic Resonance** (International Society of Magnetic Resonance, The Weizmann Institute of Science, Rehovot).

January 14–18, **6th Miami Winter Symposia** (Miami Winter Symposia, P.O. Box 906, Biscayne Annex, Miami, Florida 33152, USA).

January 16, **The Economics of Natural Resource Depletion** (D. W. Pearce, Department of Economics, University of Southampton, Southampton, SO9 5NH).

January 16, **The Nature of the Scrapie Agent** (Dr J. Barber, Botany Department, Imperial College, London SW7).

January 16–April 10, **Winter College on Surface Science** (The Deputy Director, International Centre for Theoretical Physics, P.O. Box 586, I-34100 Trieste).

January 17, **2nd National Conference on Environmental Education** (Dr J. Rose, College of Technology, Feilden Street, Blackburn, BB2 1LH, Lancashire).

January 21–23, **Hemophilia—Recent Advances in Biochemistry, Physiology, and Therapy** (Public Relations, The New York Academy of Sciences, 2 East 63rd Street, New York 10021, USA).

January 23–24, **Supramolecular Structure in Solid Polymers** (Dr E. F. T. White, Department of Polymer and Fibre Science, University of Manchester Institute of Science and Technology, Sackville Street, Manchester M60 1QD).

January 24, **Inaugural Meeting of the United Kingdom Section of the International Solar Energy Society** (Dr M. Archer, The Royal Institution, 21, Albemarle Street, London W1X 4BS).

January 29–30, **Energy: Alternatives and Risks** (Robert R. White, Director, Academy Forum, 2101 Constitution Avenue, N.W., Washington, D.C., 20418).

January 30, **Compound Semiconductor Films** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

Erratum

In the article "Refining the Earth's Pear Shape" by D. G. King-Hele and G. E. Cook (*Nature*, **246**, 86; 1973) which was published before proof corrections could be made, paragraph 1, line 7, should read "... as perigee moved successively north and south ...".

Equation (1) should read:

$$U = (\mu/r) \{ 1 - \sum_{n=2}^{\infty} J_n (R/r)^n P_n(\sin \phi) \}$$

Paragraph 8, line 1, should read "Our previous set of odd zonal harmonics, published in 1969, ...".

In reference 4, 380 should be deleted; in reference 7 delete 381.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Department of Trade and Industry. Report on Research and Development 1972-73. Pp. iii+61. (London: HMSO, 1973.) 65p net. [257]

British Association for the Advancement of Science. Annual Report, 1972/1973. Pp. 16. (London: British Association for the Advancement of Science, 1973.) [277]

Annual Report on the Meteorological Office, 1972. Pp. xii+120. (London: HMSO, 1973.) £1 net. [307]

National Radiological Protection Board, NRPB-R14: A Model for the Evaluation of the Deep Ocean Disposal of Radioactive Waste. By G. A. M. Webb and F. Morley. Pp. 23. (Harwell, Didcot: National Radiological Protection Board, 1973.) [317]

British Nuclear Fuels Limited. Second Annual Report and Accounts, 1972/1973. Pp. 24. (Risley, Warrington: British Nuclear Limited, 1973.) [18]

Energy for the Future—Report from the Working Party authorised by the Council of the Institute of Fuel. Pp. viii+28. (London: The Institute of Fuel, 18 Devonshire Street, W1, 1973.) [18]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 274, No. 1241: Numerical Solutions of the Kinematic Dynamo Problem. By D. Gubbins. Pp. 493-521. £1.10; \$3.15. Vol. 274, No. 1242: On the Kinetic Theory of Wave Propagation in Random Media. By M. S. Howe. Pp. 523-549. 95p; \$2.70. Vol. 274, No. 1243: Worldwide Distribution of Geomagnetic Tides. By S. R. C. Malin. Pp. 551-594. £1.60; \$4.50. (London: The Royal Society, 1973.) [78]

Department of Education and Science. Scientific Research in British Universities and Colleges, 1972-73. Volume 1, Part 1: Physical Sciences, Sections 1-16. Pp. xxii+1-434. Volume 1, Part 2: Physical Sciences, Sections 17-36. Pp. 435-1025. £7 net. Volume 2: Biological Sciences. Pp. xxi+809. £6 net. Vol. 3: Social Sciences (Including Government Departments and Other Institutions). Pp. xxx+751. £6 net. (London: HMSO, 1973.) [98]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 274, No. 1244: The Geology of the Bristol Channel Floor. By A. J. Lloyd, R. J. G. Savage, A. H. Stride, and D. T. Donovan. Pp. 595-626+plates 5-7. £1.75; \$4.90. Vol. 274, No. 1245: The Thermodynamics of the Titanium+Oxygen System: an Isothermal Gravimetric Study of the Composition Range TiO₂ to TiO, at 1304K. By R. R. Merritt and B. G. Hyde, and, in part, L. A. Bursill and D. K. Philip. Pp. 627-661. £1.40; \$3.90. (London: The Royal Society, 1973.) [98]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 274, No. 1246: Fixed Nuclei Two-Centre Problem in Quantum Mechanics. By J. D. Power. Pp. 663-702. £1.25; \$3.50. Vol. 275, No. 1247: On Wave-Induced Stress in a Ship Executing Symmetric Motions. By R. E. D. Bishop and R. Eatock Taylor. Pp. 1-32. (London: The Royal Society, 1973.) [298]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 265, No. 872: Organization of the Outer Synaptic Layer in the Retina of the Larval Tiger Salamander. By A. Lasansky. Pp. 471-489+plates 47-56. (London: The Royal Society, 1973.) £1.75; \$4.90. [318]

Natural Environment Research Council. Report of the Council for the period 1 April, 1972-31 March, 1973. Pp. v+168+12 plates. (London: HMSO, 1973.) £1.20 net. [189]

Proceedings of the Royal Irish Academy. Vol. 73, Section A, No. 11: The Production of Condensation Nuclei by Alpha Radiation. By T. P. Burke and J. A. Scott. Pp. 151-158. 14p. Vol. 73, Section B, No. 11: The Stratigraphy of the Middle and Upper Dalradian Succession Between Glengad Head and Greencastle, Inishowen, Co. Donegal. By J. C. Roberts. Pp. 151-164. 18p. (Dublin: Royal Irish Academy, 1973.) [209]

The Ridgeway Path. (London: The Countryside Commission, 1973.) *Gratis*. [209]

The Law of the Sea. By Elizabeth Young and Brian Johnson. (Fabian Research Series, No. 313.) Pp. 48. (London: Fabian Society, 1973.) 50p. [219]

Sub-Atomic Particles: a New Approach. By Gerald Mcateau. (Review Monograph (Physics), No. 1.) Pp. 12. (Harlow, Essex: Gerald Mcateau, 224 Nicholls Tower, 1973.) 80p. \$2. [219]

Other Countries

International Atomic Energy Agency. Annual Report, 1 July 1972-30 June 1973. Pp. 75. The Agency's Budget for 1974. Pp. 105. (Vienna: International Atomic Energy Agency, 1973.) [108]

US Environmental Protection Agency. Action for Environmental Quality. Pp. 20. (Washington, DC: US Environmental Protection Agency, 1973.) [178]

Pramana: a Journal of Physics, Vol. 1, No. 1, July 1973. Pp. 1-60. Edited by S. Ramaseshan and B. M. Udagankar. Annual subscription rates: Foreign: Institutions and Libraries \$30 or £12; individuals \$20 or £8. Domestic: Institutions and Libraries Rs.75; individuals Rs.25; members of IPA Rs.20; bona fide students Rs.10. Published monthly. (Bangalore: Indian Academy of Sciences, in collaboration with the Indian Physics Association and the Indian National Science Academy.) [218]

United States Department of the Interior: Geological Survey. Professional Paper 766: Foraminifera of the North Pacific Ocean. By Patsy B. Smith. Pp. iii+27+plates 1-4. (Washington, DC: Government Printing Office, 1973.) 85 cents. [298]

World Directory of Medical School/Répertoire Mondial des Ecoles de Médecine, 1970. Pp. 147. (Geneva: WHO; London: HMSO, 1973.) 20 Sw.francs: £2.50; \$6. [179]

Records of the Australian Museum, Vol. 28, No. 15: The Alpheid Shrimp of Australia, Part 1: The Lower Genera. By Dora and Albert H. Banner. Pp. 291-382. (Sydney: The Australian Museum, 1973.) \$4. [179]

United States Department of the Interior: Geological Survey. Bulletin 1258-E: A New Method for the Determination of Three-Dimensional Deformation Anisotropy in Rock Specimens. By Richard A. Farrow. Pp. iii+12. 25 cents. Bulletin 1372-H: Lower and Middle Tertiary Stratigraphic Units of the San Emigdio and Western Tehachali Mountains, California. By T. H. Nilsen, T. W. Dibble, Jr., and W. O. Addicott. Pp. iii+23. 35 cents. Bulletin 1376: Some Results of Geochemical Sampling in McCormick County, South Carolina. By Henry Bell, III. Pp. iv+22+plates 1-5. \$1.75. Professional Paper 532: Cenozoic Fossil Mollusks from Western Pacific Islands: Gastropods (Turritellidae Through Strombidae). By Harry S. Ladd. Pp. iv+80+plates 1-20. \$1.50. (Washington, DC: Government Printing Office, 1972 and 1973.) [249]

Publications du Centre National pour l'Exploitation des Océans (CNEXO). Série: Rapports Scientifiques et Techniques. No. 16: Etude non Linéaire des Ondes Internes dans un Milieu à Deux Couches Fluides sans Rotation. Par Alain G. Cavanic. Pp. 11. No. 17: Etude non Linéaire des Ondes Internes dans un Milieu à Deux Couches Fluides en Rotation. Par Michel Arhan. Pp. 14. (Paris: Centre National pour l'Exploitation des Océans, 1973.) [249]

Smithsonian Contributions to the Earth Sciences. No. 10: Mineralogy, Mineral-Chemistry, and Composition of the Murchison (C2) Meteorite. By Louis H. Fuchs, Edward Olsen and Kenneth J. Jensen. Pp. iii+39. 75 cents. Smithsonian Contributions to Zoology. No. 143: Bibliography and Zoological Taxa of Paul Bartsch. By Florence A. Ruhoff. With a Biographical Sketch by Harald A. Rehder. Pp. v+166. \$2.85. No. 151: Revision of Corophiidae and Related Families (Amphipoda). By J. Laurens Barnard. Pp. iv+27. 55 cents. No. 152: Evolution of the Rails of the South Atlantic Islands (Aves: Rallidae). By Storrs L. Olson. Pp. iii+53 (11 plates). 95 cents. No. 153: Western Atlantic Shrimps of the Genus *Solenocera*, with Description of a New Species (Crustacea: Decapoda: Penaeidae). By Isabel Perez Farfante and Harvey R. Bullis, Jr. Pp. 33. 65 cents. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [259]

Evolutionary Theory, Vol. 1, No. 1, July 1973. Pp. 1-50. Edited by Isidore Nahi. Subscriptions: All subscriptions from Latin America, Asia, Eastern Europe, and Africa (except South Africa and Rhodesia) \$6. Institutions elsewhere \$15. Individuals elsewhere \$10. Students \$6. Single copies \$4. (Chicago: *Evolutionary Theory*, Biology Department, 1103 E. 57th Street, The University of Chicago, 1973.) [269]

Republique Française. Office de la Recherche Scientifique et Technique Outre-Mer. ORSTOM. Travaux et Documents de l'ORSTOM. No. 19: Les Eaux du Pacifique Occidental à 170° E entre 20° S et 4° N: Coupes et Cartes par es Océanographes du Centre ORSTOM de Nouméa. Par H. Roitschi, Ph. Hisard et F. Jarrige. Pp. 113. 24 francs. No. 21: Economie Urbaine d'Antsirabe (Madagascar—1966-1969). Pp. 153. 40 francs. No. 22: Etude Ethologique et Bioéconomique du Peuplement d'Insectes dans un Verger Naturel. Par G. Couturier. Pp. 96. 30 francs. No. 23: Les Plantes Médicinales de la Nouvelle-Calédonie. Par Jean Rageau. Pp. 139. 42 francs. Mémoire ORSTOM No. 64: Ecologie de *Glossina Palpalis Gambiensis* Vanderplank, 1949. (Diptera: Muscidae) en Savane d'Afrique Occidentale. Par A. Chailier. Pp. xvi+274. 100 francs. Faune Tropicale XIX: Les Crevettes Profondes de l'Atlantique Oriental Tropical. Par A. Crosnier et J. Forest. Pp. 408. (Paris et Bondy: Office de la Recherche Scientifique et Technique Outre-Mer, 1972 et 1973.) [289]

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